Regulation of COX-2 Expression in Human Cancers

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Introduction

Metabolites of arachadonic acid participate in normal growth responses and in aberrant cellular growth and proliferation, including carcinogenesis [1, 2]. The key step in the conversion of free arachidonic acid to prostaglandins is catalyzed by the cyclooxygenase enzyme (COX). Until recently, only one isoform of the COX enzyme (COX-1) had been purified and cloned. However, it is now known that a second inducible isoform of the COX enzyme (COX-2) exists. The COX-1 enzyme is constitutively expressed at low levels in a majority of tissues and presumably makes prostaglandins for normal physiological functions. By contrast, COX-2 is not normally present in most cells, but tight regulation allows it to be rapidly expressed in response to growth-related signals resulting in increased prostaglandin synthesis associated with inflammation and carcinogenesis.

Insight into the molecular regulation of COX-2 expression preceded its discovery. Work in the late 1980s identified an inducible COX activity that was temporally regulated on both transcriptional and post-transcriptional levels [3]. Near the same time, at least two independent groups had identified and cloned COX-2 as an immediate-early response gene whose expression was highly induced in response to cellular transformation by v-src [4] or treatment of cells with phorbol ester [5]. Subsequent work led to the cloning of the human COX-2 cDNA from vascular endothelial cells [6, 7] and isolation of the COX-2 gene [8]. Examination of the COX-2 5′ promoter region has identified several transcription factor regulatory elements. A number of mRNA instability elements are contained within the 3′ untranslated region (3′UTR) of COX-2
mRNA. The fact that COX-2 expression is regulated at both transcriptional and post-transcriptional levels implies that fine control of expression is important.

The connection between COX-2 expression and carcinogenesis was first suggested by studies that demonstrated the efficacy of aspirin and NSAIDs to reduce the relative risk of colon cancer and also promote tumor regression in both humans and experimental animal models of colon cancer [2]. Investigation of the molecular basis of these observations showed 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 [9–12]. Similar to the situation with colorectal cancer, other solid malignancies such as breast, lung, prostate, pancreas, bladder, stomach, esophagus, and head and neck exhibit elevated COX-2 levels resulting from changes in the regulation of COX-2 expression. This association of COX-2 overexpression and carcinogenesis was further established in genetic studies demonstrating a significant reduction in intestinal polyposis in mice deficient for COX-2 gene [13] along with demonstration of COX-2 overexpression is sufficient to induce breast tumorigenesis [14] and pre-malignant lesions in the skin of mice [15]. Taken together, 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 or progression of human cancers.

Several lines of evidence gathered from epidemiological, experimental models, and cellular studies indicate that unregulated COX-2 expression is an important step in tumorigenesis and indicate that dysregulation occurs early in carcinogenesis, particularly colorectal cancer. The control of COX-2 expression is a complex regulatory process that requires input from multiple signal transduction pathways. Cellular defects in these signaling pathways can differentially promote the expression of COX-2 through the loss of transcriptional and post-transcriptional regulatory mechanisms. The sum of these effects results in a dramatic increase in COX-2 protein levels and associated prostaglandin production. This review is intended to summarize the major advances in the field of molecular regulation of COX-2 expression in cancer, largely focusing on the recent findings pertaining to colorectal cancer. Particular attention is paid to novel mechanisms controlling COX-2 expression at the post-transcriptional level.

**Transcriptional Regulation of COX-2**

A variety of studies support the hypothesis that an early event in tumorigenesis, such as the mutation of the ‘gate-keeper’ adenomatous polyposis coli
(APC) gene, initiates the unregulated expression of COX-2 [16, 17]. However, the molecular events leading to the constitutive transcription of COX-2 in human cancer are not totally understood. Evidence of constitutive activation of the COX-2 promoter occurring in colon cancer cells [12] suggested that the increased levels of COX-2 mRNA detected in colorectal adenomas, adenocarcinomas [9, 12] and colon cancer cell lines [12] occurs through increased transcription. Similarly, increased COX-2 transcription has been observed in transformed mammary epithelial cells [18]. Although the underlying mechanism is not completely clear, several key cis-acting elements localized within 250 bases of the transcription start site of the COX-2 gene promoter region have been shown to play a decisive role in the regulation of COX-2 expression in human carcinoma cells (fig. 1).

The Proximal Region of the COX-2 Promoter Regulates Transcription

TATA Box: A characteristic element present in many immediate-early response genes, such as COX-2, is the TATA box. Recognition of the TATA box is conferred by the TATA-binding protein (TBP) which promotes assembly of a functional transcription initiation complex. The p53 tumor suppressor protein has been demonstrated to inhibit expression of genes containing a TATA box [19] and recent findings have shown that wild-type p53 protein causes a marked decrease in COX-2 gene transcription as a result of competition for TATA-box binding with TBP [20]. This notion is supported by observations demonstrating correlation between p53 mutation and COX-2 overexpression in several human cancers [21]. Thus, the occurrence of p53 mutational inactivation, a common genetic event in human cancer [22], may prove to be an important link in understanding why constitutive expression of COX-2 occurs in many cancers.

CRE: Original observations showing the involvement of the cAMP-response element site (CRE) in COX-2 transcriptional regulation were demonstrated in cells responding to v-src activation or growth factor treatment [23, 24]. As a consequence of JNK kinase cascade activation, enhanced phosphorylation
of c-Jun occurs allowing it to activate transcription as either a homodimer or heterodimer with c-Fos or ATF-2, producing the AP-1 transcription factor. Due to altered MAPK signaling detected in colon cancer cells and transformed mammary epithelial cells, increased activation of AP-1 contributes to enhanced COX-2 gene transcription through the CRE site [25–27]. Furthermore, AP-1-mediated COX-2 transcription is an effective target of inhibition by chemopreventive agents. Studies of cancer cells treated with peroxisome proliferator-activated receptor (PPAR) ligands or retinoids display decreased COX-2 transcription as a result of their ability to squelch CBP/p300, a co-activator of AP-1 mediated gene expression which links AP-1 to the basal transcription machinery [28, 29].

**PEA3:** The transcriptional activation of COX-2 has been observed under conditions where the transcriptional activator β-catenin is stabilized as a consequence of APC mutation in intestinal tumors and Wnt signaling in breast tumors [18, 30, 31]. However, since COX-2 has not been shown to be a direct target of β-catenin-mediated transcription, this suggests that β-catenin may possibly activate COX-2 transcription through an intermediary transcription factor [32]. Recent findings have shown the PEA3 subfamily of Ets transcription factors promote COX-2 transcription in breast and colorectal cancer lines through a PEA3/Ets site present in the COX-2 promoter [27, 32]. Accordingly, high PEA3 transcription factor levels have been detected in both intestinal and breast tumors [33]. Although the connection between β-catenin and PEA3 is not known, the correlation between PEA3 and COX-2 expression in both breast and intestinal tumors suggests that PEA3 contributes to COX-2 transcriptional upregulation in response to both Wnt1 signaling and mutations in APC.

**NF-IL-6:** The trans-acting factors that promote transcription through the NF-IL-6 site include members of the CCAAT/enhancer-binding protein (C/EBP) family of basic leucine zipper transcription factors [34]. Binding of C/EBP to the NF-IL-6 site has been reported to be crucial for COX-2 promoter activation by stimuli such as mitogens and growth factors in a number of normal and carcinoma cell lines [35–39]. The NF-IL-6 site is also important for COX-2 transcription in a number of colorectal cancer cell lines [26]. Phosphorylation of C/EBP protein through a Ras-dependent MAPK cascade is essential for C/EBP activation and increases C/EBP binding to the COX-2 promoter [40, 41], suggesting that constitutive COX-2 transcription detected in colon cancer cells is influenced through enhanced C/EBP phosphorylation as a result of the presence of oncogenic Ras and increased MAPK activity [26, 42, 43]. Thus, the C/EBP proteins may regulate COX-2 gene expression in cancer cells through direct interaction with the NF-IL-6-binding site and also modulate the binding of other factors known to participate in COX-2 transcriptional activation [32, 36, 39].
**NF-κB**: A growing body of evidence indicates that NF-κB activation may play a important role in promoting COX-2 transcription in cancer associated with inflammation [44]. When exposed to inflammatory agonists, NF-κB has been shown to be a positive regulator of COX-2 transcription in human colon cancer cells [45]. These findings suggest a role for NF-κB-mediated activation of COX-2 expression in inflammatory bowel disease [46] and are of significance since chronic inflammation is a known risk factor for several types of human cancers [1].

**Post-Transcriptional Regulation of COX-2**

Messenger RNA turnover is a highly regulated process that plays a central role in the regulation of mammalian gene expression. In normal cells, the expression of growth-related gene products encoding cytokines, growth factors, and proto-oncogenes are tightly regulated via rapid decay of their respective mRNA transcripts. These mRNAs are inherently unstable due to the presence of a common cis-acting element known as the AU-rich element (ARE) which targets the mRNA for rapid decay [47, 48]. This element, most often present within the 3’ untranslated region (3’UTR) of the mRNA, can also regulate protein translation by acting as a translation inhibitory element [49–51]. The ability of the ARE to regulate gene expression can be modulated by endogenous and extracellular stimuli allowing for rapid changes in the abundance of these mRNAs when needed [47].

*The COX-2 mRNA Contains an AU-Rich RNA Element That Targets It for Rapid Decay and Translational Inhibition*

The first evidence suggesting that COX-2 might be regulated at a post-transcriptional level was the identification of multiple copies of the AUUUA sequence motif within its 3’UTR [6, 7]. In the human COX-2 gene, the 3’UTR-containing exon 10 contains 22 copies of the AUUUA sequence [8] and can be processed to yield COX-2 mRNA transcripts of multiple lengths due to alternative polyadenylation sites [52, 53]. Of particular significance was a cluster of 6 AUUUA sequence elements localized in the proximal part of the 3’UTR (fig. 2). The context of these AUUUA motifs within the 3’UTR strongly suggests the involvement of this region in regulating mRNA stability. This AU-rich region is highly conserved in both sequence and location among human, mouse, rat, chicken, pig, cow and sheep COX-2 mRNA transcripts, implying that the function of the ARE had been evolutionary conserved. Earlier investigations suggested that COX-2 expression could be regulated through its 3’UTR [52, 54]. More recent experiments have substantiated these findings by directly
showing that the COX-2 3′UTR can confer post-transcriptional regulation through rapid mRNA turnover and translational inhibition [55–60]. Post-transcriptional regulation was shown to be dependent on the COX-2 3′UTR or the conserved AU-rich region since their presence conferred rapid decay on a normally stable chimera reporter mRNA [55–57]. It should be noted that other regions within the COX-2 3′UTR have also been implicated to play a role in the regulation of COX-2 expression [60].

A number of observations suggest that defects in the ability of AREs to regulate gene expression on a post-transcriptional level play a role in neoplastic transformation of cells [61–63]. When AU-rich elements are removed from the proto-oncogenes c-fos and c-myc, there is a correlation with increased oncogenicity [64, 65] and cells show enhanced tumorigenicity when expressing IL-3 lacking the normal ARE-containing 3′UTR [66]. Additionally, a variety of human tumor cells show enhanced mRNA stability of ARE-containing cytokine genes [67] and a reporter gene containing the 3′UTR of GM-CSF is stable in monocytic tumor cells [68]. Similar findings have also been observed in human colon carcinoma cells with regard to regulation of COX-2 expression [26, 69]. Interestingly, colon cancer cells maintained the ability to rapidly degrade c-myc mRNA, suggesting that defects in rapid mRNA decay are specific to the transcript [69]. The c-myc mRNA contains a class I ARE, which is characterized by dispersed AUUUA motifs in association with stretches of U-rich regions. In contrast, COX-2 mRNA contains a class II ARE, which has multiple copies of AUUUA motifs clustered together [47]. The class II type ARE is a common feature of many growth factor mRNAs associated with angiogenesis such as VEGF and IL-8. These and other ARE-containing growth factors have been shown to be upregulated in cancer cells that have altered post-transcriptional regulation of COX-2 [26, 69].

**Fig. 2.** The human COX-2 AU-rich element. The representation of the human COX-2 mRNA is not to scale. The filled circles represent AU-rich sequences, AUUUA, contained within the 3′UTR (open bar); circles adjacent to one another indicate multiple repeat elements. The 116 nucleotide sequence of the COX-2 ARE in uppercase letters contains six AU-rich sequence motifs (AUUUA). The COX-2 termination codon is shown in lowercase letters. Solid triangles represent canonical AAUAAA polyadenylation sites; open triangles indicate non-canonical AUUAAA sites.
COX-2 Expression Is Regulated by AU-Rich RNA Element-Binding Proteins

AREs appear to mediate their regulatory function through association of multiple trans-acting RNA-binding proteins that have an affinity for the AUUUA sequence motif [47, 48]. The ability of AREs to regulate mRNA stability occurs through recruitment of a complex of RNA-degrading exonucleases termed the exosome [70, 71]. It appears that ARE-binding proteins associated with rapid decay provide a functional link between the exosome and ARE-containing mRNAs; this occurs either through its direct association within the exosome [71] or targeting of the exosome to mRNA through recruitment [70].

The COX-2 AU-rich element is also a target of cellular ARE-binding factors. Cytoplasmic proteins have been shown to form stable complexes with the COX-2 3'UTR [54, 56, 57]; the AREs from COX-2 and GM-CSF are recognized by similar RNA/protein complexes [56]. This complex, when cross-linked to the COX-2 ARE, is comprised of several distinct proteins with sizes ranging from 90 to 35 kDa [56]. It appears that these factors may play distinct roles in regulating both COX-2 mRNA stability and translation through ARE binding, since the proteins of lower mass appear to be preferentially polysome associated [56].

A number of ARE-binding factors have been identified [47, 62] and the particular factor bound to the RNA can promote rapid mRNA decay, increase mRNA stability, or regulate translational efficiency. Thus, the findings demonstrating enhanced stability of COX-2 mRNA and reporter constructs containing the COX-2 3'UTR in both colon tumor cells [26, 69] and transformed intestinal epithelial cells [42, 43, 58, 59], suggested that the observed post-transcriptional defects were a result of altered ARE recognition by trans-acting regulatory factors. Significant differences in ARE binding have been observed in the HT29 colon cancer cell line which explains the observed lack of COX-2 post-transcriptional regulation in this cell system [69]. These defects in ARE binding have also been found in other human colon cancer cells displaying increased COX-2 expression [unpublished observations]; this suggests that loss of proper ARE-recognition is a common cellular defect occurring during colon tumorigenesis. Currently, 4 factors have been identified to bind the COX-2 ARE-containing 3'UTR and influence COX-2 expression. These factors, their role in regulating COX-2 expression, and the potential importance in pathogenic states are discussed below:

HuR (HuA): The HuR protein is a ubiquitously expressed member of the ELAV (Embryonic-Lethal Abnormal Vision in Drosophila) family of RNA-binding proteins [72]. HuR protein has a high affinity and specificity for AREs and overexpression of HuR stabilizes ARE-containing mRNA transcripts...
and promotes their translation [73–75]. HuR is also a nuclear-cytoplasmic shuttling protein [76] and it is thought that the ability of HuR to promote mRNA stabilization requires its translocation to the cytoplasm [73, 74, 76].

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 [69, 77]. The enhanced stabilization of COX-2 mRNA observed in colon cancer cells is, in part, due to elevated levels of HuR, resulting in enhanced binding to the COX-2 ARE [69]. Increased levels of cytoplasmic HuR protein were detected in neoplastic epithelial cells of colon tumors [69], which may reflect its ability to promote epithelial COX-2 expression during the later stages of carcinogenesis. Accordingly, the ectopic overexpression of HuR promoted the expression of endogenous COX-2 and the ARE-containing angiogenic factors VEGF and IL-8 [69]. Through its enhanced ARE binding, HuR could indirectly inhibit COX-2 mRNA deadenylation by promoting binding of the poly(A)-binding protein to the poly(A) tail [47]. Alternatively, HuR binding may impede the formation or recruitment of the exosome to the COX-2 transcript [70, 71]. Based on the ability of HuR to shuttle between the nucleus and cytoplasm, potential alterations in the HuR-accompanied transport of COX-2 mRNA from the nucleus to the cytoplasm in tumor cells may have direct implications on COX-2 mRNA turnover and association with the translational apparatus [75]. Taken together, these findings define a role for aberrant HuR expression in colorectal neoplasia by promoting the unregulated expression of COX-2 and associated angiogenic factors similar to other pathogenic states [77–80].

**TTP (Tristetraprolin):** TTP is a member of a small family of zinc finger-containing proteins of the CCCH class [81]. Originally identified as an immediate-early response gene whose expression was transiently induced by extracellular stimuli, TTP was proposed to be a transcription factor. However, growth factor or mitogen treatment of cells resulted in the enhanced cytoplasmic localization of TTP, presumably occurring through increased nucleocytoplasmic shuttling of the protein [81, 82], suggesting an alternative role for TTP. More recently, it has been demonstrated that TTP can promote the rapid decay of ARE-containing mRNAs by directly binding to the ARE [82, 83]. The binding of TTP to AREs from inflammatory mediators, such as TNF-α and GM-CSF, targets them for rapid deadenylation and decay through exosome recruitment [70, 84]. TTP is also rapidly phosphorylated through activation of the ERK and p38 MAPK pathways by extracellular stimuli [85, 86]. It is hypothesized that the post-translational modification of TTP inhibits ARE binding, thus allowing for mRNA stabilization [85, 86].

Recent investigations in a human colon carcinoma cell line (HCA-7) have postulated that TTP is involved in the regulation of COX-2 mRNA
turnover [87, 88]. Binding of TTP to the COX-2 3’UTR has been demonstrated in conjunction with an inverse relationship between TTP mRNA and COX-2 mRNA with changes in cellular growth status. Clearly, determining the role of TTP in regulating COX-2 expression in cancer will be of great interest, since TTP deficiency in mice results in syndromes of inflammatory arthritis and bowel disease owing to defective turnover of inflammatory mediators [82, 84].

**AUF1 (hnRNP D):** The AUF1 protein was identified as part of a complex that accelerates the rapid decay of the ARE-containing c-myc proto-oncogene mRNA [89]. Through its ability to bind AREs with high affinity, AUF1 expression has been correlated with rapid mRNA decay [90]. The *AUF1* gene yields 4 protein isoforms as a result of alternative splicing and differences exist among their ability to promote rapid decay [91]. Interestingly, AUF1 can enhance mRNA stabilization under cellular responses invoked from stress and differentiation signals, suggesting that specific signals cause AUF1 to be sequestered in an inactive cytoplasmic or nuclear complex inhibiting the cytoplasmic mRNA decay function of AUF1 [92]. These types of altered cellular signaling are characteristic of cancer and thus may promote the increased cytoplasmic expression of AUF1, as detected in lung cancers [79]. Notably, the overexpression of AUF1 has been shown to promote sarcoma development in transgenic mice [93]. Presumably this increase in AUF1 expression could occur during tumor progression thereby leading to the stabilization of ARE-containing mRNAs, including COX-2, since the AUF1 protein has been demonstrated to bind the COX-2 ARE [57].

**TIA-1/TIAR:** TIA-1 protein was identified as an apoptosis-promoting factor in thymocytes and is 80% identical to that of the TIA-1-related protein, TIAR. Both factors are RNA-binding proteins and have been demonstrated to bind regions of RNA containing short stretches of uridylates [94]. Under normal conditions, both TIA-1 and TIAR are primarily localized in the nucleus [95]. Yet, both proteins are translocated to the cytoplasm in response to cellular stress signals where they co-localize with untranslated mRNAs in discrete cytoplasmic foci, implicating a role in translational regulation [95]. Consistent with this observation, both factors have been shown to specifically bind the ARE of TNF-α and can regulate its expression through translational inhibition [50, 51]. Deletion of TIA-1 in mice leads to elevated levels of TNF-α and increased sensitivity to LPS [51]. TIA-1 and TIAR interact with the COX-2 ARE [69], although the precise role of these factors in COX-2 regulation is unknown. Presumably, binding of these factors acts to repress COX-2 translation as part of a repressor complex, similar to TNF-α, since they do not appear to influence rapid COX-2 mRNA decay [unpublished observation].
Regulation of COX-2 Expression by Signal Transduction Pathways

**Multiple Signaling Pathways Are Involved in Regulating COX-2 Expression**

The regulation of COX-2 expression is a complex process involving multiple signal transduction pathways. This level of complexity underscores the requirement for tight regulation of the enzymatic action of COX-2, which has pathogenic effects if its expression is left unchecked. As a result of genetic mutations commonly detected in a majority of human tumors, cellular defects in these signaling pathways promote the expression of COX-2 through the loss of transcriptional regulation, rapid mRNA decay, and translational inhibition (fig. 3). These primary signaling pathways and their involvement in regulating COX-2 expression in human cancer are discussed below.

**Wnt/APC**: Wnt1 is a mammary oncogene encoding a secreted signaling factor and expression of Wnt1 promotes mammary carcinoma formation [96]. A downstream modulator of Wnt signaling is the tumor suppressor gene, APC. Mutations in the APC gene occur frequently in both familial and sporadic colorectal cancers [97] and these defects in APC predispose mice to intestinal and mammary tumors [13, 98]. A consequence of Wnt misexpression or APC mutations results in cytoplasmic stabilization and nuclear accumulation of the transcriptional activator β-catenin [96].

COX-2 appears to be a common downstream target of Wnt expression and APC mutations. In Wnt-1 transformed murine mammary epithelial cells, elevated COX-2 levels are detected as a result of transcriptional activation [18].

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Fig. 3. Signaling pathways regulating COX-2 expression. Schematic diagram outlining the major signal transduction pathways participating in regulation of COX-2 gene expression. Relevant signaling molecules are discussed in the text and their role in COX-2 regulation is indicated.
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 [30, 31], suggesting a possible role for β-catenin in stimulating COX-2 transcription [32]. Interestingly, the role of APC in COX-2 regulation appears to be multifaceted. In colon cancer cells containing mutations in APC, the expression of full-length APC downregulates COX-2 protein expression, while mRNA levels are unchanged [99], suggesting a link between APC and the regulation of COX-2 translation.

**Ras Signaling:** Ras proteins are membrane-bound GDP/GTP-regulated switch molecules that convert signals from the cell membrane to the nucleus. Ras mutations are found to occur in a wide variety of human malignancies. K-ras mutations are found in colon, lung and pancreatic cancers, whereas H-ras is frequently mutated in cancers of the urinary tract and bladder [100]. Approximately 50% of large colorectal adenomas contain mutant Ras, so its activation is believed to be a relatively early event in neoplastic progression [22]. Oncogenic mutations in the Ras gene lead to constitutive activation of this small GTPase resulting in activation of multiple downstream signal transduction MAPK cascades (fig. 3). With regard to regulating COX-2 expression, both oncogenic K-ras and H-ras stimulate COX-2 transcription through activation of the Rho, JNK, ERK, and p38 MAPK pathways, whereas COX-2 mRNA stability is modulated through activation of signaling pathways involving ERK, p38 MAPK, and Akt/PKB [25–27, 42, 43, 101]. Oncogenic Ras may also modulate the translation of COX-2 since it has been shown to promote phosphorylation of the translation initiation factor eIF-4e and promote enhanced protein synthesis [102].

Ras signaling has also been shown to participate with growth factors to enhance the expression of COX-2. There is mounting evidence that TGF-β can enhance malignant transformation and tumor progression for several different epithelial tumors [103] and TGF-β is abnormally expressed in over 90% of human colon cancers [104]. Accordingly, treatment of intestinal epithelial cells with TGF-β can induce or augment COX-2 expression [105–108], and recent work has demonstrated that TGF-β synergistically enhances the expression of COX-2 in Ras-transformed intestinal epithelial cells through mechanisms involving COX-2 mRNA stabilization [58].

**Rho Pathway:** The Rho family of proteins are key components of oncogenic cellular transformation of cells by Ras [109]. Rho-dependent activation of COX-2 transcription has been demonstrated in a variety of cell types, including colon and breast cancer cells, through cis-acting elements present in the proximal region of the COX-2 promoter (fig. 1) [26, 110, 111].

**JNK Pathway:** It is well documented that activation of the Ras/Rac1/MEKK1/JNK signal transduction pathway activates the transcription of COX-2.
Ras activation of the JNK kinase cascade leads to phosphorylation of c-Jun, resulting in transcriptional activation of COX-2 via the CRE element [23, 24, 36]. Increased COX-2 promoter activity, owing in part to JNK activation, has been observed in cancer cells [26, 27]. Tumor-promoting agents such as bile acids and ceramide also activate the JNK pathway leading to increased COX-2 transcription [25, 112], whereas COX-2 mRNA stability does not appear to be regulated through this pathway [59].

**ERK Pathway:** Activation of the Ras/Raf1/MEK/ERK signal transduction pathway was originally shown to modulate COX-2 transcriptional regulation in fibroblasts responding to v-src, PDGF, or serum [23, 24]. This MAPK pathway is equally important in constitutive activation of COX-2 expression in cancer. ERK activation parallels tumor progression in murine intestinal neoplasia and ERK inhibition suppresses colonic tumor growth [113]. In colorectal cancer cell lines displaying elevated levels of COX-2, constitutive activation of the ERK pathway has been observed [69] and inhibition of this pathway reduced both the COX-2 promoter activity and stabilization of its mRNA [26]. Similarly, in Ras-transformed intestinal epithelial cells, the ERK pathway is essential for Ras-mediated induction of COX-2 promoter and mRNA stabilization [42, 43, 59]. Furthermore, in HER2/neu-transformed mammary epithelial cells, HER2/neu induced COX-2 promoter activity via ERK activation [27].

**p38 MAPK Pathway:** Activation of the p38 MAPK pathway is primarily associated with cellular stress and pro-inflammatory stimuli. A number of studies have demonstrated the ability of proinflammatory signals to induce COX-2 expression in a variety of cell types through p38 MAPK activation [101, 114, 115]. Considerable evidence suggests that activation p38 MAPK promotes stabilization of COX-2 and other ARE-containing mRNAs through activation of MAPKAP-2, a kinase downstream of p38 MAPK [57, 114]. Interestingly, the ability of the anti-inflammatory glucocorticoid dexamethasone to inhibit COX-2 expression [52] has recently been shown to promote rapid destabilization of COX-2 mRNA by inhibition of the p38 MAPK pathway [116]. The ability of p38 MAPK to promote COX-2 mRNA stabilization has been detected in intestinal and breast carcinoma cells [59, 117], this may be relevant because activated p38 MAPK has been observed in neoplastic tissue [118]. p38 MAPK has also been shown to promote COX-2 transcription. In transformed mammary epithelial and cervical carcinoma cells, induction of COX-2 promoter activity was attributed, in part, to p38 MAPK activation [25, 27, 111], presumably through its ability to promote AP-1 activity via phosphorylation of ATF-2.

**Akt/PKB:** The kinase Akt, or protein kinase B (Akt/PKB), is a downstream effector of phosphophatidylinositol 3-kinase (PI3-K) [119]. Sequential activation of the PI3K/PDK/Akt/PKB kinase cascade by various growth and
survival factors promotes cell survival through inactivation of several apoptosis-associated factors. Activation of this pathway is required for Ras-mediated transformation of a variety of cell types, including intestinal epithelial cells, and Akt/PKB activation facilitates a number of cellular events associated with cellular transformation (i.e. cytoskeletal reorganization) [120, 121]. Constitutive activation of Akt/PKB has been observed in colon cancer cell lines [69] and the Akt/PKB pathway predominantly regulates COX-2 expression by modulating the stability of COX-2 mRNA [26]. In intestinal epithelial cells, oncogenic Ras-mediated post-transcriptional stabilization of COX-2 mRNA occurs through activation of the Akt/PKB pathway [43]. These findings indicate that COX-2 may represent a downstream mediator of this oncogenic pathway.

**Conclusions**

Although the molecular events leading to overexpression of COX-2 protein in human cancer have not been definitively characterized, substantial progress has been made toward understanding the mechanisms regulating COX-2 expression. Under normal cellular growth conditions, the expression of COX-2 is tightly controlled with rapid induction of COX-2 transcription occurring through a variety of stimuli. In colorectal neoplasia, a loss of transcriptional regulation causes the increased levels of COX-2 mRNA detected in a majority of colorectal adenomas, adenocarcinomas and colon cancer cell lines [9, 12, 26, 69, 122]. Notably, concomitant increase in the amounts of COX-2 protein and prostaglandins has not been observed in all adenomas or adenocarcinomas [10, 123, 124]. Moreover, increased levels of COX-2 protein were not detected all colon cancer cell lines that overexpress COX-2 mRNA [26, 69]. To reconcile the observed apparent discrepancies between dysregulated expression of COX-2 message and protein, a provisional model is presented (fig. 4). Colon cancer is used as an example, but this model could be applied to other human cancers as well. In this model, transcriptional activation of COX-2 is an early event in the initiation of a colon tumor [12, 26, 69]. However, enhanced expression of COX-2 protein also requires aberrant post-transcriptional regulation. This occurs during the transition from adenoma to carcinoma and suggests that loss of post-transcriptional regulation of COX-2 may be a crucial step in colon carcinogenesis. This notion is supported by the increase in COX-2 mRNA stability that is readily observed in colon cancer cells that constitutively express COX-2 [26, 69]. Furthermore, the observation of a correlation between increased levels of COX-2 and increased tumor size and invasiveness [13, 125, 126] suggests a link between tumor progression and defects in regulation of COX-2 gene transcription and subsequent mRNA decay. Taken together, these
control defects result in unregulated expression of COX-2 protein and presumably other immediate-early response genes that are detected in the later stages of adenoma development.

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**References**


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