Biochemical Pharmacology 80 (2010) 1801–1815

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Review

Targeting COX-2 expression by natural compounds: A promising alternative strategy to synthetic COX-2 inhibitors for cancer chemoprevention and therapy

Claudia Cerella, Cyril Sobolewski, Mario Dicato, Marc Diederich *

Laboratoire de Biologie Moléculaire et Cellulaire du Cancer, Fondation de Recherche Cancer et Sang, Hôpital Kirchberg, 9 rue Edward Steichen, L-2540 Luxembourg, Luxembourg

ARTICLE INFO

Article history:
Received 24 May 2010
Accepted 28 June 2010

Keywords:
Natural compounds
Cyclooxygenases
Cancer
Inflammation
Gene expression

ABSTRACT

Cyclooxygenase (COX)-2 is a pro-inflammatory immediate early response protein, chronically up-regulated in many pathological conditions. In autoimmune diseases, it is responsible for degenerative effects whereas in cancer, it correlates with poor prognosis. A constitutive expression of COX-2 is triggered since the earliest steps of carcinogenesis. Consequently, strategies aimed at inhibiting COX-2 enzymatic activity have been clinically applied for the treatment of autoimmune disorders; in addition, the same approaches are currently investigated for anti-cancer purposes. However, COX-2 protein inhibitors (*i.e.*, NSAIDs and COXIBs) are not amenable to prolonged administration since they may cause severe side effects, and efforts are underway to identify alternative approaches for chemoprevention/therapy. COX-2 expression is a multi-step process, highly regulated at transcriptional and post-transcriptional levels. Defects in the modulation of one or both of these steps may be found in pathological conditions. Targeting COX-2 expression may therefore represent a promising strategy, by which the same preventive and therapeutic benefits may be gained while avoiding the severe side effects of COX-2 enzymatic inhibition. Naturally occurring compounds derived from plants/organisms represent a huge source of biologically active molecules, that remains largely unexplored. Derived from plants/organisms used in traditional forms of medicine or as dietary supplements, these compounds have been experimentally investigated for their anti-inflammatory and anti-cancer potential. In this review, we will analyze how natural compounds may modulate the multistep regulation of COX-2 gene expression and discuss their potential as a new generation of COX-2 targeting agents alternative to the synthetic COX-2 inhibitors.

Contents

1. COX-2 inhibitors: limitations and alternative strategies to prevent aberrant COX-2 function

Cyclooxygenase-2 (COX-2) is one of the key enzymes implicated in the modulation of inflammation, and acts by catalyzing the

* Corresponding author at: Laboratoire de Biologie Moléculaire et Cellulaire du Cancer, Hôpital Kirchberg, 9 rue Edward Steichen, L-2540 Luxembourg, Luxembourg. Tel.: +352 2468 4040, fax: +352 2468 4060.
E-mail address: marc.diederich@lbmcc.lu (M. Diederich).

0006-2952/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2010.06.050

rate-limiting step that leads to the formation of prostaglandins (PGs) from arachidonic acid.

Two other COX isoenzymes, COX-1 and COX-3, are able to catalyze the same kind of reaction. COX-1 is the other significantly important cyclooxygenase family member, which is constitutively expressed in cells and tissues. Exact roles/functions need still to be established for COX-3, which appears expressed only in some specific compartments including brain and spinal cord [1,2].

The direct product of the activity of COX enzymes, PGH2, is in turn the precursor of other PGs, including PGE2, PGF2alpha, PGD2, PGI2 (prostacyclin) and thromboxane A2 (TxA2), through the action of cytosolic or microsomal PG synthases. Despite the fact that all the COX isoforms generate the same PGH2 precursor, and such classes of PGs have been implicated in the action of both COX-1 and COX-2, the two enzymes control very different biological processes. Whereas COX-2 is predominantly implicated in inflammation, COX-1 is a critical regulator of homeostatic functions. These differential functions may be attributed to the following molecular mechanisms. Importantly, not all the classes of PGs are necessarily produced in the same tissue and/or at the same time. The preferential production of thromboxanes due to COX-1 activity is known; COX-2 activity has been shown to lead to the accumulation of prostacyclins. These findings suggest a differential involvement of PG synthases depending on the COX isoform expressed, the mechanisms of which have yet to be elucidated. Nonetheless, specific combinations of PGs may promote different intracellular signalling cascades.

Alternatively, the modality of expression of COX-1 *vs.* COX-2 may provide further explanation about their differential functions. COX-1 is constitutively and stably expressed at low levels in many tissues. This ensures a constant production of PGs, which normally contribute to the maintenance of important physiological functions, such as platelet aggregation, renal water balance and gastric mucosal protection. In contrast, COX-2 is mostly silent but expressed in response to non-self agents, such as pathogens. When stimulated, its expression is high and transient. This leads to a burst of PG production in a tightly regulated time-limited manner. Thus, depending on the COX isoform, the production of the same earlier precursor PGH2 greatly differs in terms of amount and timing of production. This may be differentially decoded by the cells, thus leading to the activation of such intracellular pathways involving specific classes of PGs (*i.e.*, thromboxanes rather than prostacyclins) and, therefore, different responses, similar to the events observed for other pleiotropic cell signalling mediators, such as calcium ions.

The inflammatory paradigm of COX-2 expression is defined as patho-physiological, since it is part of a complex physiological self-regulating response to pathological conditions, carried on by specific cell and tissue types.

However, COX-2 expression may surpass normal physiological control, resulting in fatally pathological states: namely, autoimmune diseases and cancer. In each of these cases, an aberrant stable expression and activation of COX-2 may be occurring.

In different examples of autoimmune diseases, such as rheumatoid arthritis [3], systemic lupus erythematosus [4] or some forms of diabetes [5,6], the hyperactivation of such sub-populations of immune cells has been shown to be directly dependent on the over-expression of COX-2. The hyperexpression of COX-2 is the result of cross-talk between several mediators of inflammation, including interleukins and cytokines (*i.e.*, IL-1, IL-6 and TNFα), and it occurs *via* transcriptional activation [7].

Moreover, COX-2 has been found to be constitutively expressed in a number of cancers [8]. Epidemiological and clinical studies have shown that when COX-2 is expressed, a poor prognosis is frequently associated [8,9]. Clear evidence has been collected from models of adherent cancers, including colon [10,11], prostate [12,13] and breast [14,15], where the expression of COX-2 is paralleled by a higher incidence of chemotherapy failure. Moreover, COX-2-deficient mice are more resistant to different forms of cancers [16], as shown in models of colon [17,18] and skin carcinogenesis [19,20]. This implies a multi-step role for COX-2 in tumorigenesis, in early tumor promotion and in the late development of chemoresistance and metastatic formation [21]. Different mechanisms regulating COX-2 expression appear to be involved in this instance, with a transcriptional activation implicated in early phases, and with post-transcriptional modulation crucially contributing at late tumorigenic steps.

More recently, constitutive expression of COX-2 has been shown also to occur in haematological malignancies; again, a poor clinical outcome appears to be associated with this event [22]. Consequently, in the last decade, an upsurge of interest has arisen towards characterizing the novel potential anti-cancer therapeutic properties of modulated COX-2 functions.

So far, the clinical strategy to target COX-2 has been *via* inhibition of its activity. Non-steroidal inflammatory drugs (NSAIDs) have historically represented the first large class of inhibitors of COXs available on the market. The majority of them were used in clinics to inhibit the typical features of inflammation and pain-related symptoms even prior to the identification of the COX enzymes. Once the two COX isoforms were identified in the early 90s, it became clear that COXs were the target of NSAIDs. The elucidation of the protein sequence together with protein modelling studies of COX-1 *vs.* COX-2 showed a preferential ability of a number of NSAIDs in affecting COX-2, rather than COX-1, activity. Eventually, it was found that the catalytic active site of COX-2 was significantly larger than that of COX-1, thus generating easier accessibility for NSAIDs [23]. These findings, together with the discovery of the inducible nature of the COX-2 gene, lead to the conclusion that the anti-inflammatory properties of NSAIDs were exclusively due to COX-2 inhibition.

However, prolonged intake of NSAIDs was quickly recognized to elicit severe side effects. Studies in the 60s had already clearly identified the link between intake of salicylates and damage to the gastric mucosa [24]. The preferential, but not exclusive, action of NSAIDs on COX-2, coupled with the concomitant perturbation of COX-1 activity, was considered responsible for these alterations; this phenomenon was termed the COX-2 hypothesis [25], which exclusively assigned acute pro-inflammatory roles to the inducible COX-2 and homeostatic functions to the constitutively active COX-1.

At the end of 90s, a second generation of NSAIDs were developed based on this model; the so-called COXIBs family of drugs promised to overcome these harsh side effects [23]. Compounds in this new synthetic group are composed of two aromatic rings connected with different chemical groups which require the presence of specific residues present only in the active site of COX-2 for binding, ensuring high selectivity [23]. Their introduction to the commercial market represented an alternative to the traditional NSAIDs, which was anticipated to conjugate the use of a selective COX-2 inhibitor with the absence of severe gastrointestinal side effects. Unfortunately, an increased incidence of cardiovascular diseases was found to develop in patients exposed to a prolonged intake of some of these new agents [26]. This raised concerns about the use of COXIBs and led to their removal from the market, specifically for rofecoxib and valdecoxib; still other forms of the drugs, such as celecoxib, were subjected to intense experimental re-evaluation to determine clinical risks *vs.* benefits [27]. Such ongoing studies are still far from providing a final definitive statement. However, careful clinical analyses have indicated that restricted usage is not associated with severe side effects. As a precaution, the current guidelines for those COXIBs remaining on the market recommend limiting chronic or

prolonged administration of COX-2 inhibitors, particularly impacting their application for chemoprevention.

The cardiovascular effects associated with prolonged intake of COXIBs have been attributed to the prevention of production of the pro-vasodilator prostacyclins in the endothelial and smooth muscle cells as a consequence of COX-2 inhibition [28]. Thus, this effect is mechanism-based and implies that COX-2 is also constitutively expressed in these tissues where it controls important homeostatic functions [29]. As importantly, this finding extended our knowledge of its spatial expression pattern, leading to a significant re-evaluation of the established COX-2 hypothesis [23].

These findings support the consideration that prolonged administration of COX-2 inhibitors would be ineffectual for chemopreventive and chemotherapic purposes since the risks outweigh the benefits. Moreover, these findings raised the concern that direct COX-2 enzymatic inhibition might not represent an eligible strategy to be pursued in clinics to target COX-2, being COX-2 inhibitors unable to discriminate between an aberrant pathological *vs*. homeostatic functional activation state.

In contrast to COX-1, COX-2 is an early response gene, similar to the genes encoded for cytokines, chemokines and proto-oncogenes. Typically, these genes are subject to several mechanisms of expression modulation, ranging from direct transcriptional effects to post-transcriptional and translational levels and up to protein stability of mediating transcription factors. The presence of such multiple levels of modulation of COX-2 expression implies the existence of multiple pathways/mechanisms, which may be targeted to finely modulate COX-2 functions. This prompts the consideration of COX-2 expression as a more versatile target to modulate the wide array of its enzymatic functions, thus potentially offering new perspectives in therapeutic and chemoprevention strategies.

Natural compounds represent a huge untapped source of biologically active compounds. A keyword search of the PubMed database for relevant publications in 2009 only yields more than 6000 papers on natural compounds. A large number of these publications report anti-inflammatory and/or anti-cancer properties. Directly extracted or derived after partial chemical modification techniques, biologically active molecules from plants and micro-organisms (*i.e.*, of marine origin) represent many of the natural compounds historically used in traditional forms of medicine. This offers the advantage that many of these compounds have already been established as having a subtle or null toxicity and have been successfully used in humans. Thus, their use is established as adequately safe and provides a distinct advantage over synthetically-derived chemical substances. Being components of products frequently consumed for dietary reasons, these molecules are generally well absorbed and metabolized. Finally, most of these compounds require a relatively low cost for their extraction/production in large amounts, characterizing them as amenable to commercial objectives.

To date, a number of natural occurring compounds has been proven as inhibitors of COX-2 expression. This indicates their promise in future applications for prevention as well as for clinical therapy of COX-2-related pathologies.

2. Determinants of COX-2 expression

2.1. Transcriptional regulation

COX-2 is one of the pro-inflammatory mediators whose expression may be induced at the very early steps of carcinogenesis [30]. This requires its transcriptional activation in pre-neoplastic stages. The prevention of its aberrant expression could, therefore, translate to prevention of the formation of cancer before of its insurgence and may be a potent strategy for cancer prevention. Accordingly, a regular intake of NSAIDs has been the focus of many experimental studies and has been found to successfully reduce the incidence of colorectal cancer in subjects bearing the gene mutation responsible for familial polyadenomatosis (FAP) [31].

The transcriptional activation of the COX-2 gene has been investigated in-depth [30,32,33]. The advancements in our knowledge of this gene regulation have specifically benefited the use of a few well-established cell/tissue models of normal physiology and disease. These systems, mainly of mouse and rat origin, include *in vitro* as well as *in vivo* models of activation of COX-2 gene transcription. Appropriately stimulated with different combinations of chemicals/physical stressors, they effectively mimic the events of several physio-pathological conditions. Accordingly, the cultured murine macrophages, RAW 264.7, stimulated with LPS/INFγ have become a common model of acute inflammation [34]. The skin of mice treated with the potent chemical carcinogens phorbol 12-myristate 13-acetate (PMA) [35,36] or dimethylbenzanthracene (DMBA) [37] or exposed to ultraviolet B irradiation (UVB) [38] generates the *in vivo* prototype to study the early steps of skin carcinogenesis; used in parallel with *in vitro* models based on non-carcinogenic cell lines, such as that from the human breast MCF 10A treated with PMA [36] or the UVB-irradiated human keratinocytes HaCaT [39], these experimental systems have proved particularly informative. Moreover, these systems represent examples of tissues/cells for which the inflammatory processes accompanying aberrant COX-2 expression has been widely documented, and is expected to mimic pre-neoplastic stages. More recently, rat glial cells have been considered as a model for neuroinflammation [40,41], which contributes to determining the extent of neuronal damage in neurodegenerative diseases.

Despite the fact that these systems have largely contributed to our understanding of important modulatory aspects of COXs gene transcription, the approach relies on stimulation of the few well-known pathways, which are mainly related to a canonical activation of the nuclear factor-κB (NF-κB) pathway (see below). The COX-1 family member lacks characteristic TATA and GC boxes in its promoter region, and generally acts as a house-keeping gene; the few sequence elements for SP-1 binding have been suggested to mediate COX-1 over-expression during pro-inflammatory conditions [29]. In contrast, the COX-2 promoter contains a number of upstream regulatory sequences specific for binding with a variety of transcription factors, such as NF-κB, the SP-1 transcription factor (SP-1), the cAMP responsive element binding protein (CRE), the transcription factor 4 (TCF4), the CCAAT/enhancer-binding protein beta (c/EPB), and the activator protein 1 (AP-1) [33]. The majority of these transcription factors are pleiotropic in nature, being the final executors for a myriad of intracellular signalling pathways, whose activities may correlate to the inflammatory response. Several kinase-mediated signals may be implicated. They include those controlled by the protein kinase C (PKC); the phosphatydil-inositol-3-kinase/protein kinase B (PI3K/PKB); and the mitogen-activated protein kinases (MAPKs) c-Jun NH2-terminal kinase (JNK), p38 and the extracellular signal-regulated protein kinases 1/2 (ERK) [30,33]. Accordingly, a wide range of stimuli may trigger these intracellular signalling pathways, and downstream transcription of COX-2. Some of these pathways are known to be directly related to inflammatory conditions stimulated by pathogens, nitric oxide, cytokines, and the same PGs produced by COX-2, thus acting along a positive feedback route; still others, more diversified, include growth factors, stress factors, such as those involved in responses to irradiation, redox imbalance and mechanical shear stress, and oncogenes, such as those involved in the RAS pathway [32]. All these factors act by eliciting COX-2 transcription.

In a few cases, COX-2 expression may be transcriptionally prevented. This is the case for signalling mediated by peroxisome proliferator-activated receptors (PPARs) [42] and glucocorticoids [43]. Both factors interfere with the binding of AP-1 and NF-κB to the COX-2 promoter [32].

The presence of so many binding sites for such different transcriptional factors reflects the complexity of COX-2 transcriptional regulation. Dynamic combinations of transcriptional factors may co-operate to differentially promote COX-2 expression, depending on the cell type or the nature of the stimulus, to elicit a specific intracellular signalling response. This may offer a potential target for mediating distinct patho-physiologic modalities of COX-2 expression.

Despite a canonical activation of COX-2 gene expression *via* triggering of intracellular pathways to activate the binding of specific transcriptional factors, recent studies have indicated that COX-2 expression may be significantly modulated by chromatin remodelling events. Indeed, the 5′-flanking region of the COX-2 gene contains several cytosine-guanine-rich dinucleotides (CpG) islands, which when hypermethylated are responsible for transcriptional silencing [44,45]. Accordingly, hypomethylation of the COX-2 promoter has been found in models of gastric carcinoma [46], thus supporting the hypothesis that a loss of COX-2 promoter methylation may play a key role in gastric carcinogenesis [45]. However, no hypermethylating agents are currently available as a strategy to regulate aberrant COX-2 expression. Moreover, changes in acetylation status of histones and non-histone proteins have been identified as an alternative epigenetic event and similarly implicated in the constitutive activation of the COX-2 gene, with events of hyperacetylation promoting COX-2 expression [47]. Agents hampering the level of histone acetylation, such as histone deacetylase inhibitors (HDACs), have been shown to suppress COX-2 expression in cancer cell models [47]. However, data so far collected are in favour of an indirect effect of HDAC inhibitors, where their real target is not the COX-2 promoter but the promoters of genes coding for transcription factors that regulate the COX-2 promoter, *i.e.*, c-Jun [47] and further studies are required to prove an actual role of HDAC inhibitors.

The alterations induced by the methylation status of chromatin proteins may affect the expression of the genes in multiple ways. Directly, by modulating the three-dimensional conformation of chromatin and by promoting the binding of free pools of transcriptional factors. Indirectly, by interfering with the binding of transcription suppressors and by making the promoter accessible for the transcriptional factors; alternatively, by modulating the expression of regulatory factors required to activate the COX-2 promoter. NF-κB, for example, triggers COX-2 expression early in the pre-neoplastic stages, and has been suggested as a factor whose expression may be modulated by altered methylation status [48].

2.2. Post-transcriptional regulation

2.2.1. Control of COX-2 mRNA stability and translational inhibition by AU-rich elements (AREs)

Immediate-early response genes have evolved a sophisticated system of post-transcriptional control of their expression, aimed at ensuring a stringent time-limited production of the proteins they encode. This system implies the existence of early mechanisms which determine a rapid decay and/or a translational inhibition of the mRNA transcripts and involve *cis*- and *trans*-acting elements operating in a concerted fashion. On one side, the mRNA transcripts of these classes of genes possess a 3′-untranslated region (3′-UTR) which contains a number of copies of the highly conserved *cis*-acting consensus motif AAUAAA (or strict close variants of it), generally referred as AU-rich elements or AREs [49]. On the other side, several proteins are known to recognize and bind these regions, the AREs-binding factors, to *trans*-modulate the physical accessibility and stability of the target mRNAs for translation [49,50]. COX-2, as well as other pro-inflammatory genes, is known to undergo this kind of modulation. This form of translational control is currently hypothesized as a central, possibly decisive, checkpoint during the acute inflammatory response. Barik and co-workers [51] have recently speculated that it may represent an interesting system to rapidly produce inflammatory-related modulators during the early phase of acute inflammation. Likewise, the signal may be robustly and immediately switched off before it becomes detrimental. This intriguing scenario implies that alterations in the 3′-UTR-based mechanisms may be responsible for many instances of chronic inflammation. Besides, when constitutively activated, they might act as a promoting mechanism of carcinogenesis.

Unlike COX-1, COX-2 mRNA possesses a complex 3′-UTR, containing multiple polyadenylation signals. Two of them appear to be the most used, and are commonly referred to as the proximal and distal signals, in accordance with their relative distance from the stop codon. Consequently, two main COX-2 transcripts, strikingly different in size (2.8 and 4.6 kb, respectively), may be formed [32]. A bioinformatic approach, confirmed by *in vivo* studies, has indicated that the distribution of the alternate COX-2 transcripts is tissue-specific, with the usage of the distal polyadenylation signal occurring predominantly in colon, lung and epithelial tissues [52]. The usage of either polyadenylation signal depends on specific nucleotide variances in the proximal consensus sequences, which render the proximal site sub-optimal. Site-direct mutagenesis of the proximal consensus motif increases its usage, and results in the formation of the shorter COX-2 transcript [52]. Further upstream *cis*-acting auxiliary elements known as the upstream efficiency elements (USEs), exist and modulate the efficiency of polyadenylation at the proximal site by recruiting specific *trans*-acting protein factors [52,53]. To-date, the physio-pathological outcomes of these alternate COX-2 transcripts have not yet been elucidated. However, some theories have been put forth. Several cancer cell types, commonly used as a model for deciphering pro-carcinogenic roles of COX-2, such as the colorectal cancer model HT-29, produce long transcripts [52]. The distance between the proximal and the distal polyadenylation signal is about 2000nt, and contains at least 22 identified additional AREs [54], which are targets of additional factors modulating mRNA silencing [49,54]. Finally, the shorter and the longer transcript have been shown to present a differential half-life in different instances [54,55]. However, such an unequivocal relationship may not be assigned to the size of the transcript and its half-life. The presence of the same sequences upstream of the COX-2 proximal polyadenylation signal has been shown to confer increased [49] or reduced [50] mRNA stability, depending on the studies. All these pieces of evidence, even if conflicting, are strongly suggestive of a direct role played by the additional 3′-UTR in conferring a differential mRNA stability. Moreover, it should be taken into account that however mRNA stabilization takes place, this does not imply that the downstream event of translation may also be favoured. For example, in epithelial cells the CUG-binding protein 2 (CUGBP2) act as a *trans*-acting regulatory factor, binding to AREs within the COX-2 3′-UTR following stress signals. Once bound, it potently inhibits COX-2 mRNA translation, even if it stabilizes and increases the half-life of COX-2 transcripts [56].

Besides, it is conceivable that the choice of one or the other polyadenylation signal, as well as the nature of the modulation they exert, may reflect different metabolic status as well as states of tissue differentiation, possibly mediating the recruitment of different *trans*-acting protein complexes. From this point of view, a future challenge will be to identify appropriate cell models to

elucidate whether alternate COX-2 transcripts might be differentially produced depending on the physiological (*i.e.*, homeostatic) *vs.* physio-pathological (*i.e.*, inflammatory *vs.* carcinogenic) roles played by COX-2. This will provide important information about whether in transformed *vs.* normal tissues a differential abundance of the shorter *vs.* longer COX-2 transcript form may be preferred. These insights will also contribute to identify important factors regulating aberrant COX-2 expression, possibly revealing novel agents that mediate this level of COX-2 expression.

The HT-29 cell line represents a commonly used cancer cell model for which a causative role of COX-2 is well documented in early stages of cancer progression [10]. A comparison of the mRNA transcript decay levels in COX-2-expressing colon cancer cell lines and healthy primary cells with COX-2-induced expression (*i.e.*, primary human monocytes stimulated with LPS) immediately shows a striking stability of COX-2 mRNA in cancer environments, with a half-life of hours *vs.* a few minutes in the healthy cells [50,57]. Dixon et al. have shown that this type of deregulation depends on a cluster of six AREs residing in the proximal region of the 3′-UTR targeted by a complex of *trans*-acting RNA binding factors whose expression appears to be altered in colon cancer cells [50]. Moreover, these authors demonstrated that the same region of the 3′-UTR contributes to the success of COX-2 mRNA translation [50]. By elucidating the dynamic modulation of these factors and the effects that this modulation may exert on COX-2 expression, a particularly high potential for preventive and therapeutic approaches has been realized. Four RNA binding proteins have been implicated as crucial to this mechanism [57–59]. Two of them determine opposite effects on mRNA stability in a dynamic equilibrium; consequently, any aberrant expression of one of these two factors may unbalance this delicate form of control, thus leading to an uncontrolled COX-2 mRNA stabilization.

The protein HuR, which positively mediates COX-2 expression in colon cancer cell lines [57], is also known to promote stabilization of COX-2 mRNA by protecting the poly(A) tail from degradation. Accordingly, ectopic over-expression of HuR by a tetracycline-regulated expression system in COX-2-negative cells promoted and stabilized endogenous COX-2 expression [57]. Other studies, performed on different forms of cancer, including uterine, cervical and breast [60,61], have further confirmed a causative association between HuR up-regulation and COX-2 over-expression. This prompted the consideration of HuR over-expression as a general biomarker of carcinogenesis related to aberrant COX-2 expression, and supports targeting of HuR as an attractive potential strategy for future cancer therapies. The action of HuR, however, is counteracted by tristetraproline (TTP) [58], a factor promoting mRNA decay of a number of pro-inflammatory mediators, including TNFα and GM-CSF, through exosome recruitment [62,63]. HuR and TTP recognize and bind a sequence region of multiple AREs that partially overlap [64], thus creating a competive binding inhibition mechanism. Interestingly, TTP has been found to be down-regulated in colon cancer [65]. The concomitant HuR over-expression dramatically shifts the balance towards a stabilizing effect of COX-2 transcripts. The same altered balance has been suggested to exist in other tumors [66,67]. Finally, TTP deficiency has been implicated in promotion of several autoimmune diseases in mouse models [68], thus confirming the general relevance of this dynamic double control system in pathology.

Dixon et al. have also shown that the proximal 3′-UTR is also targeted by the other two common ARE-binding proteins, the T cell intracellular Ag-1 (TIA-1) and the TIA-1-related protein (TIAR), which control the efficiency of translation [59,69]. TIA-1 and TIAR bind regions of RNA containing short stretches of uridylates [70] and work downstream to HuR/TTP, by acting as translational silencers of COX-2 expression; accordingly, their deficiency has been proved in functional studies as causative for elevated levels of TNFα and COX-2 [59,69,71].

Alterations in the level of expression of some of these factors are only part of the malignant aberrations that may be found. HuR is, for example, a well-known target of the stress-related kinases MAPK p38 [39,72] and PKCs isoforms [72,73]. HuR phosphorylation promotes its translocation to the cytoplasm [72]; furthermore, it contributes to increases in the binding affinity of HuR to mRNA [72,73]. The relevance of post-translational modifications in modulating *trans*-acting elements responsible for COX-2 mRNA stability offers interesting points of discussion about the multi-step action of such kinases in gene expression.

The 3′UTR of COX-2 mRNA contains also some consensus binding elements specific for the eukaryotic translation initiation factor 5A-1 (eIF-5A) [74]. This factor has been implicated in RNA turnover [75]. To become functional, it undergoes post-translational modifications mediated by polyamines, a pool of molecules that appears aberrantly abundant in cancer patients [76]. The chemopreventive agent DL-alpha-difluromethylornithine (DMFO), which causes polyamines depletion, has been shown to increase COX-2 steady state RNA levels, thus suggesting that polyamines may paradoxically negatively regulate COX-2 and that the use of DMFO should be thought in combination with COX-2 inhibitors to magnify the different anti-cancer mode of actions of both molecules [74,77]. However, the actual role of polyamines on COX-2 mRNA stability/translation is still far from a definitive statement.

Taken together, all these findings stress the relevance of the modulator roles played by specific ARE clusters contained within the 3′-UTR of COX-2 mRNA and identify some of the *trans*-acting proteins binding to this region as promising targets to mediate transcript stability and translation involved in the formation of some cancers.

2.2.2. Control of COX-2 mRNA stability and translational inhibition by microRNAs

Recent studies have revealed that the 3′-UTR of COX-2 mRNA transcripts may be potently regulated by another class of *trans*-acting elements, namely the small noncoding RNAs (generally referred as microRNAs or miRNAs). This broad group of RNA molecules binds to partially complementary sequences in the 3′-UTR of a number of genes, the miRNA response element (MRE) and produces a dual modulatory mechanism of mRNA turnover and translation in the same way as AREs-binding proteins. Their recent discovery has revolutionized the knowledge about the actual impact of post-transcriptional modulation in gene expression, thus leading to a critical re-evaluation of the necessity of other upstream regulatory mechanisms of gene expression. Consequently, significant efforts are being currently applied towards their identification. These intensive investigations have so far contributed to the discovery of a number of miRNAs, which appear to be implicated in regulating cellular processes such as cell proliferation, differentiation, metastasis formation, and apoptosis—all crucial events affected in cancer. Accordingly, miRNAs are currently suggested as a further class of tumor suppressors or oncogenes.

Microarray analyses together with *in vitro* studies have contributed to the identification of a first panel of miRNAs implicated in the post-transcriptional control of COX-2 as well as of other pro-inflammatory mediators. The first microRNA directly implicated in the modulation of COX-2 expression was miR-101a [78,79]. Such homeostatic processes as embryo implantation [79], and mammary tissue development and time-limited differentiation occurring during lactogenesis [80], where transient COX-2 expression occurs, have provided the first models for the characterization of the role played by this small non-coding

RNA. In both processes, a conserved inverse relationship between the level of expression of miR-101a and COX-2 may be consistently found, with up-regulation of miR-101a regularly fitting with COX-2 silencing. The data so far collected are in favour of an inhibition of translational events, as opposed to an increase in COX-2 mRNA decay [78]. Subsequently, miR-101a has been characterized as down-regulated in patients affected by endometrial serous adenocarcinoma and colorectal forms of cancer [81]. Furthermore, functional studies based on the restoration of miR-101a expression have confirmed a significant reduction of COX-2 protein expression [80].

More recently, a correlation has been found between the expression of other microRNAs and COX-2. miR-26b was examined in a model of naso-pharyngeal carcinoma [82] and miR-16 in tumor monocytes [83]. Luciferase constructs containing sub-regions of the COX-2 3′-UTR evidenced a direct ability of these miRNAs to bind to the COX-2 3′-UTR [78,82]; in addition, the up-regulation of each specific micro-RNA corresponded to the down-regulation of COX-2 protein.

Many of the *trans*-acting proteins that bind to AREs within the COX-2 3′-UTR may be in turn regulated at the post-transcriptional level by such microRNAs. HuR, for example, is a known target of both miR-519 [84,85] and miR-125a [86]. Accordingly, their aberrant down-regulation has been associated with HuR over-expression in a number of cancers, mainly classified as adenocarcinomas, where HuR up-regulation is purported to be a prognostic factor [81,87]. Interestingly, this dual inverse altered pattern of expression of microRNAs/HuR correlated with the deregulated increase of cell proliferation, a phenomenon that is commonly associated with COX-2 over-expression in cancer [9,84,86].

This scenario is further complicated by the observation that such factors that normally bind the COX-2 promoter and trigger COX-2 gene transcription may be regulated by miRNAs. miR-155 [88], miR-9 [89] and miR-146 [90], for example, have been implicated in modulating the NF-κB pathway *via* translational events.

These findings delineate the existence of a delicate, still largely unexplored, dynamic interplay, modulated by a number of microRNAs, which may act by controlling COX-2 expression through multiple mechanisms in a direct as well as an indirect fashion.

2.3. Post-translational modulation

It has emerged that COX-2 protein may be subjected to different post-translational modifications. They include events of N-glycosylation [91–94]; S-nitrosylation [95]; phosphorylation [96]; and acetylation [97]. Whether these modifications might play a role in cancer, it is not clear. However, strong evidence of a role played by these post-translational events in modulating COX-2 protein stability/enzymatic activity is emerging. This prompts to investigate the impact of this final step of COX-2 expression modulation in carcinogenesis in the future. Results may imply the potential identification of novel chemopreventive agents acting through the modulation of the post-translational modifications.

COX-2, as well as COX-1, undergoes three events of N-glycosylation at the level of specific residues of asparagine (Asn) [91]. They take place concomitantly with the translation and are absolutely required for the acquisition of the correct protein folding permitting the downstream insertion of the heme group, the formation of the dimer and the insertion into the membranes of the endoplasmic reticulum of the mature protein [91]. Thus, they are essential for the functionality of COXs enzymes. The modified residues appear highly conserved in COX-1 and COX-2 amino acid sequence [91,93].

These events of N-glycosylation are responsible for the gain of the molecular weight of the COXs protein, which from the complete inactive aglycosylated form of 66 kDa, detectable with the inhibitor of N-glycosylation tunicamycin [98], reaches the molecular weight of the active 72 kDa form.

COX-2, which possesses a 60% of homology in the sequence with COX-1, differs from COX-1 for the presence of an additional C-terminal sequence of 19aa. Within this sequence, a fourth residue of asparagine (Asn-580) may be a further target of N-glycosylation [91,92]. This modification does not occur systematically, thus excluding a direct role in modulating COX-2 enzyme activity [91,92]. The variability of N-glycosylation at Asn-580 is responsible for the production of two different glyco-isoforms of COX-2, respectively of 72 and 74 kDa [98]. Sevigny et al. [92] have provided first evidence that this modification is mostly important for the protein turn over. Through experiments of mutagenesis, consisting in the replacement of asparagine with glutamine, they proved that the removal of the glycosylation site increased the total COX-2 protein. This modification had also an impact on the total activity that is higher in the mutated COX-2 form [92]. Mbonye et al. have confirmed these findings [93], providing strong evidence about the existence of a proteasomal pathway which is triggered upon the post-translational N-glycosylation and which is modulated by an instability 27 amino acid motif upstream of the N-glycosylated site [93]. Altogether, these findings have shed light on the biological significance of COX-2-specific modifications, thus indicating a further element of instability in COX-2 expression, operating directly at COX-2 protein level.

The proteasomal pathway is part of a more complex modulatory network controlling COX-2 protein half-life. Mbonye et al. have described, indeed, in the same study an alternative pathway of COX-2 protein degradation activated by the substrate and consisting in an irreversible suicide inactivation due to the damage of the catalytic site [93] (further details may be found also in a review from the same group [94]).

Besides, studies performed on cells of the nervous system have shown that COX-2 may be subjected to S-nitrosylation [95] and phosphorylation [96]. Whereas N-glycosylation occurs on newly synthesized COX-2 protein molecules [98], these latter modifications target pre-existing COX-2 proteins, as demonstrated by the use of cycloheximide [95,96]. S-nitrosylation appears due to the direct interaction between neuronal nitric oxide synthase (nNOS) and COX-2 enzyme and leads to an increased COX-2 activity [95]. Tyrosine phosphorylation also fits with an increased COX-2 activity in neuronal endothelial cells [96]. Accordingly, the tyrosine kinase inhibitors genistein and tyrphostins reduced COX activity, whereas phosphatase inhibitor phenylarsine oxide, vanadate and benzylphosphonic acid further increase COX activity [96]. Interestingly, both these modifications have been so far detected in endothelial-neuronal tissues, where a constitutive expression of COX-2 may be found. Thus, they may be relevant for the characterization of neuro-inflammatory-mediated diseases; moreover, they may also provide new elements characterizing the differential homeostatic *vs*. pathological expression of COX-2.

Finally, acetylation may occur in the catalytic site of COX enzymes [99]. This modification is responsible for the inactivation of the COXs and represents the mechanism of action of aspirin, the only non-steroidal anti-inflammatory drug that operates by covalently modifying COXs [99]. Very recently, this kind of modification has received increased interest. In the emerging scenario of a complex interplay existing between COXs, LOXs and lipid metabolism mediating inflammation, it has been found that COX-2, once activated, may also trigger the production of downstream oxidative metabolites of omega-3 poly-unsaturated fatty acids (PUFAs), which participate to the resolution of the inflammation [100,101]. This implies a dual role for COX-2 during inflammation, thus COX-2 promoting both initiation and termination of the inflammatory response. When COX-2 is acetylated, the

anti-inflammatory function of the enzyme appears particularly exacerbated [100]. At the light of these very recent findings, some attempts based on the design of aspirin-like molecules, but presenting a preferential activity on COX-2 enzyme, might be re-evaluated for therapeutic purposes [97].

3. Modulation of COX-2 expression by natural compounds

3.1. COX-2 transcriptional regulation by natural compounds

The availability of such established cell/tissue models, where COX-2 transcription may be easily triggered, has contributed to the identification of a number of natural occurring compounds acting at this level of COX-2 gene expression (Fig. 1). Those plants extensively used in traditional forms of medicine as well as those used for dietary purposes, whose regular consumption correlated with a lower incidence of cancer, have represented the first natural arsenal from which to extract potentially therapeutically bioactive molecules. Table 1 highlights the natural compounds known to affect COX-2 expression.

Polyphenols represent the largest source of phytochemicals with promising chemopreventive and chemotherapeutic potential. Abundantly present in plants and fruits, this group is subdivided among several sub-classes characterized for their anti-inflammatory and anti-cancer properties (see Table 1). In particular, the NF-κB inflammatory mediator has been identified as a target of many polyphenols [30]; our lab has recently identified several of these novel natural inhibitors of the NF-κB pathway [102–104]. Moreover, these molecules have been characterized for their ability to affect COX-2 enzyme functions [30] *via* transcriptional control of COX-2 gene expression. The inhibition of the NF-κB pathway or the perturbation of such kinase-dependent signalling events, *i.e.*, those mediated by JNK, p38 or ERK, has been widely depicted as the main mechanism [105].

Curcumin, a polyphenol mainly extracted by the rhizomes of the turmeric *Curcuma longa*, is one of the major phytochemicals characterized for its anti-inflammatory and anti-cancer properties [106]. Initially described for its ability to suppress PG production [107], it has become clear that this compound plays multiple roles towards COX-2 regulation, and directly prevents COX-2 gene expression [107]. The analysis of the effects of curcumin on a wide variety of cell models, appropriately stimulated to induce COX-2 expression, has lead to the converging observation that the transcriptional machinery controlling COX-2 expression is affected. The NF-κB pathway, in particular, appears to be strongly inhibited, as evidenced in *in vitro* systems of stabilized cancer cell lines as well as in *in vivo* mouse tumor models [108,109]. Accordingly, the prevention of different NF-κB-related events has been described, including the nuclear translocation of NF-κB subunits (*i.e.*, p65), the DNA binding activity and the degradation of the inhibitor IκBα [108,110]. Similar inhibitory effects of curcumin have been shown in relation to AP-1 [109,111], which also binds COX-2 promoter. In many of these systems, curcumin prevents the activation of various MAP kinases. This is the case for JNK and p38 in human keratinocytes [111] and rat models of experimental colitis [112]. Besides, the ability of curcumin to prevent PMA-induced phosphorylation of ERK1/2 and p38 MAPK has been observed in the skin carcinogenetic mouse model [108,111]. The activation of these kinases operates upstream of NF-κB and AP-1, thus indicating an ability of curcumin to act at very early steps in the stress signalling responses that lead to COX-2 expression [108,110–112].

Curcumin can also act through another mechanism. For example, curcumin is able to inhibit the expression of the transcription factor Ets-1 in human endometrial carcinoma HEC-1-A cells [113], which is described as a positive regulator of COX-2 transcription [114].

Besides curcumin, the phytoalexin resveratrol and the flavonoid epigallocathechin gallate (EGCG) represent the most extensively investigated polyphenols for which anti-cancer and chemopreventive roles have been well documented.

Mainly present in red wine and grapes, resveratrol has been evaluated for its chemopreventive effects by Pezzuto et al. using

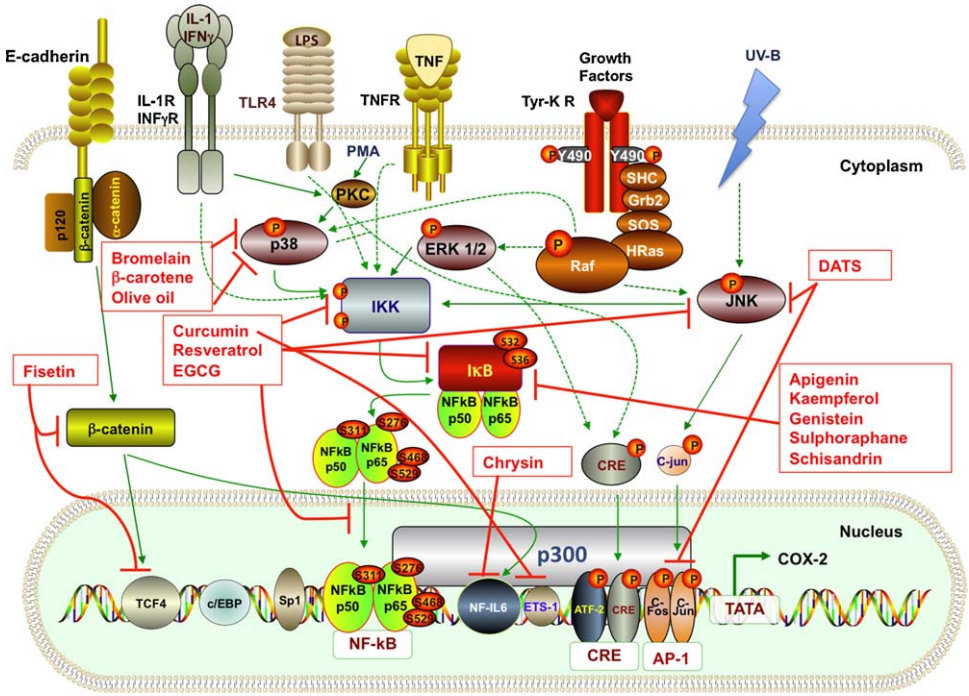

Fig. 1. COX-2 transcriptional regulation by natural compounds. A simplified version of the complex intracellular signalling network mediating COX-2 gene transcription (green arrows), showing the intracellular pathways known to be affected by natural compounds (red solid lines). Green dashed arrows indicate when some steps in the cascade events were omitted. In most cases, the effects of natural compounds are due to the inhibition of the activity of different kinases, which in turn control pathways implicated in the modulation of a variety of transcriptional factors, including the key factors NF-κB and AP-1.

Table 1
Naturally occurring compounds affecting COX-2 expression.

Natural compounds	Contained in	Biological effects on COX-2 expression	Tissues/cells models	Concentrations tested	References
Polyphenols					
Curcumin (non-flavonoid polyphenol)	Turmeric *Curcuma longa*	Block of the nuclear translocation of NF-kB subunits (*i.e.*, p65); inhibition of the NF-kB DNA binding activity; prevention of the degradation of the inhibitor IkBa	Mouse skin; Rat microglia; Human primary oral prelimagnant cells	1–25 μM; 2–16 μM; 50 μM	[108–110]
		Inhibition of the AP-1 DNA binding activity	Human gastrointestinal cancer cell lines Rat microglia Human keratinocytes	0.5–20 μM 2–16 μM 5–10 μM	[106,109,110]
		Prevention of JNK, p38 MAPK, ERK1/2 activation	Mouse skin; Human keratinocytes; Colon	1–25 μM; 5–10 μM; 50–100 mg/kg	[108,111,112]
		Down-regulation of Ets-1 transcription factor	Endometrial adenocarcinoma	16–60 μM	[113]
Resveratrol (non-flavonoid polyphenol; phytoalexin)	Red wine; grapefruit	Inhibition of the NF-kB DNA binding activity	Mouse skin	25–50 mg/kg	[35]
		Inhibition of the AP-1 DNA binding activity	Human mammary epithelial cells; Human lymphoma	5–100 μM ; 0.1–25 μM	[118]
		Prevention of PKC, p38 MAPK, ERK1/2 activation	Human mammary epithelial cells	2.5–30 μM	[105,116]
Epigallocathechin gallate (EGCG) (catechin)	Green tea	Inhibition of the NF-kB DNA binding activity	Murine macrophages	5–15 μM	[119]
		Inhibition of the AP-1 DNA binding activity	Human keratinocytes	5.45 nM–54.5 μM	[120,105]
		Prevention of PKC, p38 MAPK, ERK1/2 activation	Mouse skin; Human mammary epithelial cells	20–50 mg/kg; 5–100 μM	[36]
		Increased COX-2 mRNA decay (HuR down-regulation; TTP up-regulation)	Colon carcinoma; Human promyelocitic cells Rat liver/muscles	50–300 μM; 0.3–30 μM 1–2 g/kg	[133–135]
Apigenin (flavone)	Chamomile	Prevention of the IkBoc degradation *via* inhibition of IkB kinase (IKK)	Murine macrophages	1–25 μM; 3–100 μM	[122,123]
		COX-2 mRNA stabilization; translation inhibition (increased HuR/TIAR shuttling)	Murine keratinocyte	50 μM	[133]
Genistein (isoflavone)	Lupin; fava beans; soybean	Prevention of IkBoc degradation *via* inhibition of IkB kinase (IKK)	Murine macrophages	1–25 μM	[122]
Kaempferol (flavonoid)	Tea; broccoli; grapefruit; apple; brussel sprouts	Prevention of IkBoc degradation *via* inhibition of IkB kinase (IKK)	Murine macrophages	1–25 μM	[122]
Chrysin (flavonoid)	Honey; propolis	Inhibition of NF-IL-6 DNA binding activity	Murine macrophages	10 μM	[125]
Fisetin (flavonoid)	Onion; cucumber; apple; persimmon; strawberry	Inhibition *via* phosphorylation of the (3-catenin pathway	Colon Carcinoma	60–120 μM	[126]
Sulfur compounds					
Sulforaphane	Garlic; onion	Inhibition of the NF-kB DNA binding activity	Various Leukemia/Carcinoma cell lines	5–20 μM	[127]
Diallyl trisulfide (DATS)	Garlic; onion	Inhibition of PMA-dependent activation of c-jun and c-fos; reduction of JNK activity and AKT phosphorylation.	Mouse skin	25 μM	[37]
Miscellaneous phytochemicals					
Bromelain (protease enzyme)	Pineapple stems	Inhibition of the phosphorylation of NF-kB p65/relA; activation of AKT phosphorylation; inhibition of ERK1/2 activation	Mouse skin	52 μg–1 mg/animal	[129]
Schisandrin	Fruit of the five taste (*Schisandra chinensis*)	Inhibition of IkB degradation; inhibition of LPS-induced phosphorylation of JNK and p38 MAPK	Mouse macrophages; Mouse skin	10–50 μM; 100–200 mg/ml	[130]

Hydroxytyrosol, tyrosol and secoiridoid oleuropein	Olive oil	Inhibition of p38 MAPK and CREB phosphorylation	Human colorectal adenocarcinoma	10–100 ng/ml	[131]
Mangiferin (glucosylxanthone)	Mango tree (*Mangifera indica L.*)	Reduced COX-2 mRNA stability	Rat microglia	1–50 μM	[40]
Omega-3 fatty acids	Oily fish Flaxseeds	Inhibition of COX-2 activity COX-2 protein downregulation	Human colorectal carcinoma	60–100 μM (DHA)	[137]
			Prostate carcinoma hepatocellular carcinoma	1.73–0.247 g/day; 1–200 μM (DHA, EPA)	[140,141–143]
			Mouse skin	1%g/v (ETA)	[142]
Glucosamine	Crustaceans exoskeleton Fungi	Impairment of N-glycoylation Inhibition of COX-2 activity COX-2 protein downregulation	Human lung, bronchial, laryngeal cells	0.01–5 μM	[98]
			Human skin fibroblasts keratinocytes	10 μM	[145]

mouse models of carcinogenesis; their findings indicated an ability of this compound to prevent skin tumor formation [115]. Further studies have confirmed the ability of resveratrol to inhibit COX-2 transcription and activity in a mammary epithelial cell model mimicking tumor promotion *via* PMA-treatment [116].

EGCG, particularly abundant in green tea, was initially proposed as having anti-cancer properties based on several epidemiological studies showing an inverse correlation between a regular consumption of tea and the incidence of different forms of cancer in humans [117]. The anti-cancer potentials of EGCG have been directly correlated to the ability of EGCG to repress COX-2 expression, as confirmed in *in vivo* models of tumor promotion using PMA-treated mouse skin and in the *in vitro* human non-carcinogenic mammary cell line MCF 10A [36].

The mechanisms of action of resveratrol and EGCG appear very similar to those of curcumin. Indeed, the ability of these compounds to affect COX-2 transcription has been linked to the perturbation of the activities of NF-κB and AP-1 [35,36,118–120]. A large body of evidence, furthermore, has clearly indicated the inhibition of kinase-dependent signalling events, including those mediated by PKCs, ERK or p38 MAP kinases, as the early molecular events modulated by resveratrol and EGCG [105]. The presence of a resorcin-type moiety may play an active role in the chemopreventive potentials. Accordingly, different polyphenolic natural compounds presenting this structure have been found amongst the most active suppressors of COX-2 promoter activity in colon cancer cells [121].

A study from Liang et al. [122], has shown that the flavone apigenin and the related flavonoids genistein and kaempferol are potent transcriptional inhibitors of COX-2 in mouse macrophages RAW 264.7 stimulated with LPS or INFγ [122]. The mechanism of action occurs *via* the perturbation of the NF-κB pathway, and as a consequence of these compounds preventing the degradation of the IκBα inhibitor, due, in turn, to the inhibition of IκB kinase (IKK) activity. Similar results have been obtained in a study from Woo et al. [123] with a derivative of apigenin (2′,8″-biapigeninin), extracted from the plant Selaginella tamariscina, which is a principal constituent of oriental medicines with anti-inflammatory capabilities.

Apigenin derivatives are abundant in chamomile. In a study from Srivastava et al. [124], aqueous chamomile extracts were reported as able to reduce the levels of both COX-2 mRNA and protein in the RAW 264.7 murine macrophages. This compound did not affect COX-1 expression. HPLC analysis of chamomile extract revealed the presence of two major compounds, apigenin 7-O glucoside and apigenin 7-O neohespridoside, responsible for these biological activities.

Besides flavones, flavonoids have also been described as inhibitors of COX-2 expression. Chrysin, a flavonoid from honey, and propolis inhibit LPS-induced COX-2 expression at both the mRNA and protein levels [125]. One study revealed decreased COX-2 promoter activity due to an inhibition of DNA binding activity of the transcriptional binding factor NF-IL-6. Fisetin, a flavonoid found normally in onion, cucumber, apple, persimmon and strawberry, has been shown to reduce the level of COX-2 expression in the colon cancer cell line HT29 by inhibiting the β-catenin pathway [126]. Indeed, fisetin increases the phosphorylation of β-catenin, thereby leading to its proteasomal degradation. The ultimate consequence is an inhibition of the translocation of this protein to the nucleus together with its functional partner Tcf-4. Moreover, the fisetin compound is also able to reduce the expression of Tcf-4 itself. These two effects together inhibit the transcription of COX-2.

In addition to the polyphenols, a broad spectrum of phytochemicals with different chemical and functional properties is known to affect COX-2 expression at the transcriptional level.

Sulfur compounds, derived from garlic or onions, represent the largest group. Sulforaphane, a biologically active compound extracted from cruciferous vegetables inhibits DNA binding activity of NF-κB in several cell types, including THP-1, U937 (human monocytic leukemia), HL-60 (human acute myeloblastic leukemia), K562 (human erythroid chronic myeloid leukemia), PC-3 (human prostate carcinoma), MCF-7 (human breast carcinoma) and Hep-G2 (human hepatoma) cell lines [127]. In this study, the inhibition of NF-κB in THP-1 is due to the inhibition of TNF-α-dependent IκBα degradation and, therefore, leads to inhibition of p65 nuclear translocation and the reduction of COX-2 transcription.

Other phytochemical compounds from garlic appear to also have COX-2 transcription inhibition potential. A study from Shrotriya et al. demonstrated that diallyl trisulfide (DATS) suppressed PMA-induced COX-2 expression in mouse skin [37]. This effect was correlated with an inhibition of PMA-dependent activation of c-jun and c-fos, paralleled by a reduction of JNK activity and AKT phosphorylation. In this study, the authors hypothesized that DATS modified the cystein residues present in the DNA binding domains of c-jun and c-fos, thus leading to the inactivation of AP-1.

It has been recently suggested that bromelain, a molecule from pineapple stems, is able to decrease DMBA-induced COX-2 transcription by inhibiting the phosphorylation of p65/relA of NF-κB in RAW 264.7 macrophage cells [128]. This effect is attributable to an inhibition of the activating phosphorylation of AKT. Moreover, bromelain is also able to inhibit ERK activation, another positive regulator of COX-2 transcription. An inhibition of ERK phosphorylation has been observed in another study focused on β-carotene [129]. This molecule reduces the transcription of COX-2 in the human colon adenocarcinoma LS-174 cell line, stimulated with heregulin-α, a growth factor, which activates HER2/HER3 receptors and, thus, the MAPK pathway.

Schisandrin suppresses COX-2 transcription by inhibiting IκB degradation, leading to an inhibition of NF-κB. This molecule also acts to inhibit LPS-induced phosphorylation of JNK and p38 MAPK in RAW 264.7 macrophage cells [130].

Finally, olive oil extract, which is mainly composed of hydroxytyrosol, tyrosol and secoiridoid oleuropein, inhibits Caco-2 cell growth. This effect is related to a reduction of COX-2 transcription through an inhibition of p38 MAPK and CREB phosphorylation [131].

The natural compounds mentioned in this section appear to affect COX-2 expression at the transcriptional level. Interestingly, all of them work by perturbing cell signalling events mediated by kinases and the NF-κB/AP-1 system, aberrant activation of which characterizes the earliest steps of carcinogenesis. This strongly supports the feasibility and necessity of future efforts to evaluate the use of different naturally occurring compounds to determine their applicability in chemoprevention.

3.2. COX-2 post-transcriptional regulation by natural compounds

It is becoming clear that aberrations in mRNA stability and in translation efficiency play a determinant role in stabilizing COX-2 expression in different pathological conditions, from autoimmune diseases to cancers. For example, colorectal cancer models, which represent the best documented forms of cancer for which the carcinogenic roles of COX-2 have been ascertained, typically show increased COX-2 mRNA stability. This makes the post-transcriptional modulation of COX-2 a promising future target in clinics and prompts investigations into new and previously unrecognized agents that can act to regulate this level of COX-2 gene expression.

To-date, very few studies have been published dealing with compounds regulating this level of COX-2 expression. Likewise, cell models and experimental approaches specifically aimed at verifying any modulation of the functions of COX-2 transcripts has also been very limited. The belief is that when concerted efforts are put forth to assay naturally occurring compounds for their ability to regulate COX-2 gene expression new effective regulatory molecules identified; moreover, these types of studies will lead to a critical re-evaluation of the mechanism of action of such phytochemicals, so far assumed to act exclusively at the transcriptional level. Undoubtedly, this will provide important additional details on the post-transcriptional modulation of COX-2.

The case of apigenin and EGCG are particularly intriguing (Fig. 2). Tong et al. [132] showed that the flavone apigenin, known as a modulator of COX-2 transcription, was able to exert specific effects on COX-2 mRNA, by reducing the efficiency of COX-2 mRNA translation in a mouse model of keratinocytes irradiated with UVB. This mechanism was found to be associated with the massive translocation of the *trans*-acting translational silencer TIAR to the cytosol. Interestingly, the authors also found that the translocation to the cytosol of HuR, which normally stabilizes COX-2 transcripts, was increased by apigenin. Accordingly, apigenin increased COX-2 mRNA stability. This seemingly paradoxical effect is not new in the known functions of COX-2 transcripts, since the 3′-UTR RNA-binding protein CUGBP2 had been previously shown to increase COX-2 mRNA stability while inhibiting its translation [56]. However, this finding was suggestive of more complicated levels of regulation of COX-2 mRNA functions, which still need to be elucidated.

Peng et al. have found that the green tea polyphenol EGCG can affect COX-2 mRNA stability in colorectal cancer, in addition to reducing the activity of the COX-2 promoter (Fig. 2) [133]. This activity depends on the ability of EGCG to modulate the level of expression of the *trans*-acting elements HuR/TTP. The mRNA stabilizing protein HuR is indeed down-regulated by EGCG [134]; in contrast, the mRNA destabilizing factor TTP has been found to be up-regulated [135]. This suggested that the actual mechanism of action of natural compounds targeting COX-2 expression encompasses a multi-step modulatory process.

It is conceivable that when more studies are conducted to analyze the effects of curcumin on COX-2 mRNA stability or translation efficiency a similar pattern of alterations will be observed. Some pieces of evidence encourage this hypothesis. For example, curcumin and resveratrol prevent p38 MAPK activation [35,111]. The aberrant activation of p38 promotes COX-2 expression, playing a multi-step modulator role. It functions upstream of COX-2 transcription, by controlling NF-κB/AP-1 activation [111]. It also operates downstream, by phosphorylating HuR [39]. This activity promotes nuclear-cytoplasmic shuttling of HuR, thus enhancing COX-2 mRNA stabilization [72].

The strong effects that such natural compounds have on COX-2 expression may, therefore, be the result of multiple inhibitory effects converging at both transcriptional and post-transcriptional levels. It is conceivable that natural agents may exist that exclusively modulate the post-transcriptional events. In line with this hypothesis is the finding that mangiferin, a glucosylxanthone abundant in the bark of the mango tree (*Mangifera indica* L.) drastically reduces the stability of COX-2 transcripts without modifying the efficiency of COX-2 transcription in LPS-stimulated microglial cells [40].

COX-2 mRNA functions are regulated by *trans*-acting proteins binding to AREs or mRNAs recognizing the consensus sequence MREs. The first data to support such an ability to affect microRNAs levels are starting to be collected for several natural agents. Intriguingly, curcumin, resveratrol and EGCG are among those agents (see the very recent review from Sarkar and colleagues [87]). However, to-date, the classes of microRNAs found to be modulated by natural agents do not correspond to the particular ones that have been directly implicated in COX-2 post-transcrip-

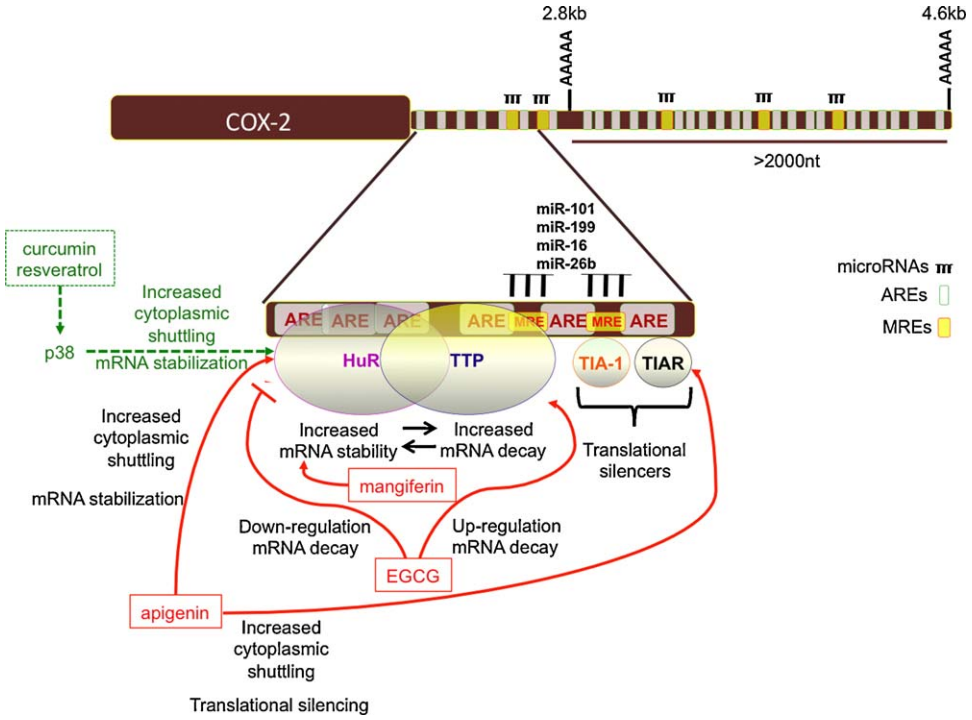

Fig. 2. COX-2 post-transcriptional regulation by natural compounds. COX-2 transcripts present two alternative sites of polyadenylation, generally referred to as proximal and distal, leading to the formation of transcripts of 2.8 and 4.6 kb, respectively. The long transcript contains an additional sequence of >2000nt comprising 22 known additional AU-rich elements (AREs). The exact roles played by these two isoforms have not yet been elucidated. The proximal 116nt region containing 6 AREs has, however, been characterized as a modulator of mRNA turnover and translation [50]. The *trans*-acting factors HuR and TTP compete for binding to partially overlapping sequences, thus promoting mRNA stabilization or decay depending on their abundance and level of activity. The two translational silencers TIA-1 and TIAR recognize and bind sequences contained in the same regions, as do some microRNAs. All these factors are believed to form a multimeric protein complex [49]. Effects of natural compounds are indicated by solid red lines with arrowheads (positive) or capped (negative). The green dashed lines indicates a suggested model of action through which curcumin may post-transcriptionally affect COX-2.

tional control, with the exception of miR-16 which has also been implicated in controlling the Bcl-2 apoptosis related family members [136].

Investigations focusing on the mechanisms of micro-RNAs and COX-2 are still in their infancy. The identification of the comprehensive miRNA cohort is still in progress, and our knowledge about the impact of each on COX-2 expression is likewise limited. To date, only a few miRNAs have been directly implicated in modulation of COX-2. Moreover, it is likely that additional miRNAs exist that may control COX-2 in an indirect manner, such as *trans*-acting elements controlling COX-2 mRNA functions. The potential of these unknown functions and molecules encourage speculation that micro-RNA regulation may represent a future strategy by which to target COX-2 expression.

3.3. COX-2 post-translational modulation by natural compounds

The discovery and the investigation of post-translational modifications that COX-2 may undergo is still a very recent field. Consequently, the knowledge about the physiological meaning the majority of them may play is still to be ascertained, especially in carcinogenesis. However, some preliminary findings encourage us to think that some chemopreventive agents acting at this level of COX-2 expression might be identified among natural products.

An intriguing case concerns the omega-3 fatty acids. Mainly contained in cold water oily fish or in plants such as flaxseeds, nowadays they are also present as dietary supplements in other foods, due to their generally accepted preventive effects on the cardiovascular diseases.

A large body of evidence is accumulating also for an anti-cancer effect of omega-3 fatty acids. The two main 3-PUFAs docosahexaenoic acid (DHA) and eicosaopentainoic acid (EPA) have been found to exert anti-proliferative effects and/or to impact cell viability in cancer models, including colon [137–139], prostate [140], and hepatocellular carcinomas [141]. Some investigations point out the chemopreventive roles of 3-PUFAs [138–140,142]. For example, an epidemiological study has established an inverse correlation between the frequency to develop aggressive forms of prostate cancer and the regular dietary consumption of 3-PUFAs [140]. This suggests an ability of 3-PUFAs to reduce chronic inflammation, which are believed one of the main pro-carcinogenic factors for prostate cancer. Besides, studies based on skin carcinogenesis models have shown that the administration of DHA, EPA or the omega-3 fatty acid 11,14,17-eicosatrienoic acid (ETA) [142] suppresses the expression of pro-inflammatory mediators and prevents skin damages. In all reports, the targeting of COX-2 functions has been found. Interestingly, both inhibition of COX-2 activity and downregulation of COX-2 expression appear to be targeted by 3-PUFAs treatment [137,139,143].

Omega-6 and -3 fatty acids represent potent mediators in the cellular lipid metabolism, which, in turn, orchestrates a complex inflammatory signalling network [138], composed of different actors [101]. The recent findings that omega-3 fatty acids may be released during inflammation and contribute to the resolution of the inflammatory response with a mechanism exactly controlled by COX-2 [100] may offer novel insights for multiple roles of COX-2 during inflammation; at the same time, they provide an interesting tool to target COX-2 protein for anti-cancer purposes.

Besides 3-PUFAs, another molecule of potential interest is the amino sugar glucosamine. Naturally present in the exoskeletons of crustaceans and in some fungi, it is a product frequently used in alternative forms of medicine for the treatment of osteoarthritis, where its beneficial effect is associated to its property to prevent cartilage degeneration, being the precursor for some major

cartilage constituents. Osteoarthritis is one of the chronic inflammatory diseases best characterized for a determinant role of COX-2 [144]. The recent finding showing that glucosamine hydrocloride impairs the N-glycosylations of COX-2 [98], therefore, blocking its activity and affecting also its stability [145], is suggestive of specific anti-inflammatory properties exhibited by this naturally occurring molecule, of potential interest for cancer chemoprevention.

4. Conclusions

Aberrant COX-2 expression is commonly found in different pathologies, where it may play a significant role in disease onset and progression. The fact that COX-2 is expressed at the earliest stages of carcinogenesis makes the targeting of COX-2 a favourable strategy for chemopreventive and chemotherapeutic purposes. Among the pro-inflammatory enzymes COX-2 has the highest number of specific inhibitors available on the market; however, the clinical use of non-selective and selective COX-2 inhibitors has revealed that inhibition of COX-2 enzymatic activity is not amenable to long-term treatment regimens [29]. Development of severe disorders, such as gastrointestinal bleeding or thrombosis and stroke, has impeded their chronic usage for preventive and therapeutic purposes.

On the other hand, the complex multi-step regulation of COX-2 gene expression offers a valid alternative to the challenges of COX-2 enzymatic inhibition and may be a preferable strategy for targeting COX-2 to treat pathological conditions. This method is especially plausible since mediators of COX-2 expression are often altered in their expression or activity in autoimmune diseases and in COX-2 expressing cancers.

Although several determinants of COX-2 gene regulation have been identified and the involved mechanisms have begun to be delineated, further studies are required. Of particular challenge will be the elucidation of the differential mechanisms regulating COX-2 homeostatic gene expression *vs.* aberrant expression in pathological conditions. This will provide answers to important questions not yet clarified, such as defining the role played by alternative COX-2 transcripts comprised of differential 3′-UTRs. Moreover, these studies will contribute to our knowledge of the differential impact achieved by modulation of the transcriptional *vs.* post-transcriptional steps of COX-2 gene expression in physiopathological conditions.

In this context, those naturally occurring compounds that target COX-2 gene expression may play a dual role. On one side, they may represent a novel class of COX-2-targeting molecules to be exploited for preventive and therapeutic purposes, due to their low or null toxicity. On the other side, they may represent a suitable tool by which to decipher the underlying molecular aspects contributing to the complexity of COX-2 gene regulation.

Acknowledgements

C.C. and C.S. are recipients of postdoctoral and doctoral Télévie grants, respectively. Research at the Laboratoire de Biologie Moléculaire et Cellulaire du Cancer (LBMCC) is financially supported by the Recherche Cancer et Sang foundation, the Recherches Scientifiques Luxembourg association, the Een Haerz fir kriibskrank Kanner association, the Action Lions Vaincre le Cancer association, and by Télévie Luxembourg. Manuscript editing costs were covered by the Fonds National de la Recherche (FNR), Luxembourg.

References

[1] Kis B, Snipes JA, Isse T, Nagy K, Busija DW. Putative cyclooxygenase-3 expression in rat brain cells. J Cereb Blood Flow Metab 2003;23:1287–92.
[2] Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci U S A 2002;99:13926–31.
[3] Martel-Pelletier J, Pelletier JP, Fahmi H. Cyclooxygenase-2 and prostaglandins in articular tissues. Semin Arthritis Rheum 2003;33:155–67.
[4] Zhang L, Bertucci AM, Smith KA, Xu L, Datta SK. Hyperexpression of cyclooxygenase 2 in the lupus immune system and effect of cyclooxygenase 2 inhibitor diet therapy in a murine model of systemic lupus erythematosus. Arthritis Rheum 2007;56:4132–41.
[5] Giulietti A, van Etten E, Overbergh L, Stoffels K, Bouillon R, Mathieu C. Monocytes from type 2 diabetic patients have a pro-inflammatory profile, 1,25-Dihydroxyvitamin D(3) works as anti-inflammatory. Diabetes Res Clin Pract 2007;77:47–57.
[6] Beyan H, Goodier MR, Nawroly NS, Hawa MI, Bustin SA, Ogunkolade WB, et al. Altered monocyte cyclooxygenase response to lipopolysaccharide in type 1 diabetes. Diabetes 2006;55:3439–45.
[7] Mino T, Sugiyama E, Taki H, Kuroda A, Yamashita N, Maruyama M, et al. Interleukin-1alpha and tumor necrosis factor alpha synergistically stimulate prostaglandin E2-dependent production of interleukin-11 in rheumatoid synovial fibroblasts. Arthritis Rheum 1998;41:2004–13.
[8] Cao Y, Prescott SM. Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. J Cell Physiol 2002;190:279–86.
[9] Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. Int J Cell Biol 2010;2010:215158.
[10] Brown JR, DuBois RN. COX-2: a molecular target for colorectal cancer prevention. J Clin Oncol 2005;23:2840–55.
[11] Ogino S, Kirkner GJ, Nosho K, Irahara N, Kure S, Shima K, et al. Cyclooxygenase-2 expression is an independent predictor of poor prognosis in colon cancer. Clin Cancer Res 2008;14:8221–7.
[12] Cai Y, Lee YF, Li G, Liu S, Bao BY, Huang J, et al. A new prostate cancer therapeutic approach: combination of androgen ablation with COX-2 inhibitor. Int J Cancer 2008;123:195–201.
[13] Aparicio Gallego G, Diaz Prado S, Jimenez Fonseca P, Garcia Campelo R, Cassinello Espinosa J, Anton Aparicio LM. Cyclooxygenase-2 (COX-2): a molecular target in prostate cancer. Clin Transl Oncol 2007;9:694–702.
[14] Zhao X, Goswami M, Pokhriyal N, Ma H, Du H, Yao J, et al. Cyclooxygenase-2 expression during immortalization and breast cancer progression. Cancer Res 2008;68:467–75.
[15] Sucic M, Boban D, Markovic-Glamocak M, Jakic-Razumovic J, Vrbanec D, Ries S, et al. Expression of cyclooxygenase-2 in fine-needle aspirates from breast carcinoma and benign breast diseases. Breast 2003;12:51–7.
[16] Langenbach R, Loftin C, Lee C, Tiano H. Cyclooxygenase knockout mice: models for elucidating isoform-specific functions. Biochem Pharmacol 1999;58:1237–46.
[17] Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, et al. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 1996;87:803–9.
[18] Chulada PC, Thompson MB, Mahler JF, Doyle CM, Gaul BW, Lee C, et al. Genetic disruption of Ptgs-1, as well as Ptgs-2, reduces intestinal tumorigenesis in Min mice. Cancer Res 2000;60:4705–8.
[19] Tiano HF, Loftin CD, Akunda J, Lee CA, Spalding J, Sessoms A, et al. Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal differentiation and reduces mouse skin tumorigenesis. Cancer Res 2002;62:3395–401.
[20] Akunda JK, Chun KS, Sessoms AR, Lao HC, Fischer SM, Langenbach R. Cyclooxygenase-2 deficiency increases epidermal apoptosis and impairs recovery following acute UVB exposure. Mol Carcinog 2007;46:354–62.
[21] Sarkar FH, Adsule S, Li Y, Padhye S. Back to the future: COX-2 inhibitors for chemoprevention and cancer therapy. Mini Rev Med Chem 2007;7:599–608.
[22] Bernard MP, Bancos S, Sime PJ, Phipps RP. Targeting cyclooxygenase-2 in hematological malignancies: rationale and promise. Curr Pharm Des 2008;14:2051–60.
[23] Marnett LJ. The COXIB experience: a look in the rearview mirror. Annu Rev Pharmacol Toxicol 2009;49:265–90.
[24] Davenport HW. Damage to the Gastric Mucosa: Effects of Salicylates and Stimulation. Gastroenterology 1965;49:189–96.
[25] Seibert K, Masferrer JL. Role of inducible cyclooxygenase (COX-2) in inflammation. Receptor 1994;4:17–23.
[26] Gottlieb S. COX 2 inhibitors may increase risk of heart attack. BMJ 2001;323:471.
[27] De Smet BD, Fendrick AM, Stevenson JG, Bernstein SJ. Over and underutilization of cyclooxygenase-2 selective inhibitors by primary care physicians and specialists: the tortoise and the hare revisited. J Gen Intern Med 2006;21:694–7.
[28] Funk CD, FitzGerald GA. COX-2 inhibitors and cardiovascular risk. J Cardiovasc Pharmacol 2007;50:470–9.
[29] Rouzer CA, Marnett LJ. Cyclooxygenases: structural and functional insights. J Lipid Res 2009;50 Suppl::S29–34.
[30] Surh YJ, Kundu JK. Signal transduction network leading to COX-2 induction: a road map in search of cancer chemopreventives. Arch Pharm Res 2005;28:1–15.
[31] Eisinger AL, Prescott SM, Jones DA, Stafforini DM. The role of cyclooxygenase-2 and prostaglandins in colon cancer. Prostaglandins Other Lipid Mediat 2007;82:147–54.

[32] Tanabe T, Tohnai N. Cyclooxygenase isozymes and their gene structures and expression. Prostaglandins Other Lipid Mediat 2002;68–69:95–114.
[33] Chun KS, Surh YJ. Signal transduction pathways regulating cyclooxygenase-2 expression: potential molecular targets for chemoprevention. Biochem Pharmacol 2004;68:1089–100.
[34] Lee SK, Hong CH, Huh SK, Kim SS, Oh OJ, Min HY, et al. Suppressive effect of natural sesquiterpenoids on inducible cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) activity in mouse macrophage cells. J Environ Pathol Toxicol Oncol 2002;21:141–8.
[35] Kundu JK, Chun KS, Kim SO, Surh YJ. Resveratrol inhibits phorbol ester-induced cyclooxygenase-2 expression in mouse skin: MAPKs and AP-1 as potential molecular targets. Biofactors 2004;21:33–9.
[36] Kundu JK, Na HK, Chun KS, Kim YK, Lee SJ, Lee SS, et al. Inhibition of phorbol ester-induced COX-2 expression by epigallocatechin gallate in mouse skin and cultured human mammary epithelial cells. J Nutr 2003;133:3805S–10S.
[37] Shrotriya S, Kundu JK, Na HK, Surh YJ. Diallyl trisulfide inhibits phorbol ester-induced tumor promotion, activation of AP-1, and expression of COX-2 in mouse skin by blocking JNK and Akt signaling. Cancer Res 2010;70: 1932–40.
[38] Kundu JK, Chang EJ, Fujii H, Sun B, Surh YJ. Oligonol inhibits UVB-induced COX-2 expression in HR-1 hairless mouse skin—AP-1 and C/EBP as potential upstream targets. Photochem Photobiol 2008;84:399–406.
[39] Fernau NS, Fugmann D, Leyendecker M, Reimann K, Grether-Beck S, Galban S, et al. Role of HuR and p38MAPK in ultraviolet B-induced post-transcriptional regulation of COX-2 expression in the human keratinocyte cell line HaCaT. J Biol Chem 2010;285:3896–904.
[40] Bhatia HS, Candelario-Jalil E, de Oliveira AC, Olajide OA, Martinez-Sanchez G, Fiebich BL. Mangiferin inhibits cyclooxygenase-2 expression and prostaglandin E2 production in activated rat microglial cells. Arch Biochem Biophys 2008;477:253–8.
[41] Gong Y, Xue B, Jiao J, Jing L, Wang X. Triptolide inhibits COX-2 expression and PGE2 release by suppressing the activity of NF-kappaB and JNK in LPS-treated microglia. J Neurochem 2008;107:779–88.
[42] Subbaramaiah K, Lin DT, Hart JC, Dannenberg AJ. Peroxisome proliferator-activated receptor gamma ligands suppress the transcriptional activation of cyclooxygenase-2. Evidence for involvement of activator protein-1 and CREB-binding protein/p300. J Biol Chem 2001;276:12440–8.
[43] Newton R, Seybold J, Kuitert LM, Bergmann M, Barnes PJ. Repression of cyclooxygenase-2 and prostaglandin E2 release by dexamethasone occurs by transcriptional and post-transcriptional mechanisms involving loss of polyadenylated mRNA. J Biol Chem 1998;273:32312–21.
[44] Harper KA, Tyson-Capper AJ. Complexity of COX-2 gene regulation. Biochem Soc Trans 2008;36:543–5.
[45] Song SH, Jong HS, Choi HH, Inoue H, Tanabe T, Kim NK, et al. Transcriptional silencing of Cyclooxygenase-2 by hyper-methylation of the 5' CpG island in human gastric carcinoma cells. Cancer Res 2001;61:4628–35.
[46] Akhtar M, Cheng Y, Magno RM, Ashktorab H, Smoot DT, Meltzer SJ, et al. Promoter methylation regulates Helicobacter pylori-stimulated cyclooxygenase-2 expression in gastric epithelial cells. Cancer Res 2001;61:2399–403.
[47] Yamaguchi K, Lantowski A, Dannenberg AJ, Subbaramaiah K. Histone deacetylase inhibitors suppress the induction of c-Jun and its target genes including COX-2. J Biol Chem 2005;280:32569–77.
[48] Deng WG, Zhu Y, Wu KK. Up-regulation of p300 binding and p50 acetylation in tumor necrosis factor-alpha-induced cyclooxygenase-2 promoter activation. J Biol Chem 2003;278:4770–7.
[49] 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;276:23179–85.
[50] Dixon DA, Kaplan CD, McIntyre TM, Zimmerman GA, Prescott SM. Post-transcriptional control of cyclooxygenase-2 gene expression, The role of the 3'-untranslated region. J Biol Chem 2000;275:11750–7.
[51] Mazumder B, Li X, Barik S. Translation control: a multifaceted regulator of inflammatory response. J Immunol 2010;184:3311–9.
[52] Hall-Pogar T, Zhang H, Tian B, Lutz CS. Alternative polyadenylation of cyclooxygenase-2. Nucleic Acids Res 2005;33:2565–79.
[53] Hall-Pogar T, Liang S, Hague LK, Lutz CS. Specific trans-acting proteins interact with auxiliary RNA polyadenylation elements in the COX-2 3'-UTR. RNA 2007;13:1103–15.
[54] Newton R, Seybold J, Liu SF, Barnes PJ. Alternate COX-2 transcripts are differentially regulated: implications for post-transcriptional control. Biochem Biophys Res Commun 1997;234:85–9.
[55] Lukiw WJ, Bazan NG. Cyclooxygenase 2 RNA message abundance, stability, and hypervariability in sporadic Alzheimer neocortex. J Neurosci Res 1997; 50:937–45.
[56] Mukhopadhyay D, Houchen CW, Kennedy S, Dieckgraefe BK, Anant S. Coupled mRNA stabilization and translational silencing of cyclooxygenase-2 by a novel RNA binding protein, CUGBP2. Mol Cell 2003;11:113–26.
[57] Dixon DA, Tolley ND, King PH, Nabors LB, McIntyre TM, Zimmerman GA, et al. Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. J Clin Invest 2001;108: 1657–65.
[58] Boutaud O, Dixon DA, Oates JA, Sawaoka H. Tristetraprolin binds to the COX-2 mRNA 3' untranslated region in cancer cells. Adv Exp Med Biol 2003;525: 157–60.
[59] Dixon DA, Balch GC, Kedersha N, Anderson P, Zimmerman GA, Beauchamp RD, et al. Regulation of cyclooxygenase-2 expression by the translational silencer TIA-1. J Exp Med 2003;198:475–81.
[60] Lim SJ, Kim HJ, Kim JY, Park K, Lee CM. Expression of HuR is associated with increased cyclooxygenase-2 expression in uterine cervical carcinoma. Int J Gynecol Pathol 2007;26:229–34.
[61] Denkert C, Weichert W, Winzer KJ, Muller BM, Noske A, Niesporek S, et al. Expression of the ELAV-like protein HuR is associated with higher tumor grade and increased cyclooxygenase-2 expression in human breast carcinoma. Clin Cancer Res 2004;10:5580–6.
[62] Deleault KM, Skinner SJ, Brooks SA. Tristetraprolin regulates TNF TNF-alpha mRNA stability via a proteasome dependent mechanism involving the combined action of the ERK and p38 pathways. Mol Immunol 2008;45:13–24.
[63] Hau HH, Walsh RJ, Ogilvie RL, Williams DA, Reilly CS, Bohjanen PR. Tristetraprolin recruits functional mRNA decay complexes to ARE sequences. J Cell Biochem 2007;100:1477–92.
[64] Anant S, Houchen CW. HuR and TTP: two RNA binding proteins that deliver message from the 3' end. Gastroenterology 2009;136:1495–8.
[65] Young LE, Sanduja S, Bemis-Standoli K, Pena EA, Price RL, Dixon DA. The mRNA binding proteins HuR and tristetraprolin regulate cyclooxygenase 2 expression during colon carcinogenesis. Gastroenterology 2009;136: 1669–79.
[66] Sanduja S, Kaza V, Dixon DA. The mRNA decay factor tristetraprolin (TTP) induces senescence in human papillomavirus-transformed cervical cancer cells by targeting E6-AP ubiquitin ligase. Aging (Albany NY) 2009; 1:803–17.
[67] Brennan SE, Kuwano Y, Alkharouf N, Blackshear PJ, Gorospe M, Wilson GM. The mRNA-destabilizing protein tristetraprolin is suppressed in many cancers, altering tumorigenic phenotypes and patient prognosis. Cancer Res 2009;69:5168–76.
[68] 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;95:1891–9.
[69] Piecyk M, Wax S, Beck AR, Kedersha N, Gupta M, Maritim B, et al. TIA-1 is a translational silencer that selectively regulates the expression of TNF-alpha. EMBO J 2000;19:4154–63.
[70] Dember LM, Kim ND, Liu KQ, Anderson P. Individual RNA recognition motifs of TIA-1 and TIAR have different RNA binding specificities. J Biol Chem 1996;271:2783–8.
[71] Gueydan C, Droogmans L, Chalon P, Huez G, Caput D, Kruys V. Identification of TIAR as a protein binding to the translational regulatory AU-rich element of tumor necrosis factor alpha mRNA. J Biol Chem 1999;274:2322–6.
[72] Jin SH, Kim TI, Yang KM, Kim WH. Thalidomide destabilizes cyclooxygenase-2 mRNA by inhibiting p38 mitogen-activated protein kinase and cytoplasmic shuttling of HuR. Eur J Pharmacol 2007;558:14–20.
[73] Doller A, Huwiler A, Muller R, Radeke HH, Pfeilschifter J, Eberhardt W. Protein kinase C alpha-dependent phosphorylation of the mRNA-stabilizing factor HuR: implications for posttranscriptional regulation of cyclooxygenase-2. Mol Biol Cell 2007;18:2137–48.
[74] Parker MT, Gerner EW. Polyamine-mediated post-transcriptional regulation of COX-2. Biochimie 2002;84:815–9.
[75] Chen KY. An 18000-dalton protein metabolically labeled by polyamines in various mammalian cell lines. Biochim Biophys Acta 1983;756:395–402.
[76] Bachrach U. Polyamines and cancer: minireview article. Amino Acids 2004;26:307–9.
[77] Reddy BS. Strategies for colon cancer prevention: combination of chemopreventive agents. Subcell Biochem 2007;42:213–25.
[78] Strillacci A, Griffoni C, Sansone P, Paterini P, Piazzi G, Lazzarini G, et al. MiR-101 downregulation is involved in cyclooxygenase-2 overexpression in human colon cancer cells. Exp Cell Res 2009;315:1439–47.
[79] Chakrabarty A, Tranguch S, Daikoku T, Jensen K, Furneaux H, Dey SK. MicroRNA regulation of cyclooxygenase-2 during embryo implantation. Proc Natl Acad Sci U S A 2007;104:15144–9.
[80] Tanaka T, Haneda S, Imakawa K, Sakai S, Nagaoka K. A microRNA, miR-101a, controls mammary gland development by regulating cyclooxygenase-2 expression. Differentiation 2009;77:181–7.
[81] Hiroki E, Akahira J, Suzuki F, Nagase S, Ito K, Suzuki T, et al. Changes in microRNA expression levels correlate with clinicopathological features and prognoses in endometrial serous adenocarcinomas. Cancer Sci 2010;101: 241–9.
[82] Ji Y, He Y, Liu L, Zhong X. MiRNA-26b regulates the expression of cyclooxygenase-2 in desferrioxamine-treated CNE cells. FEBS Lett 2010;584:961–7.
[83] 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;283:36221–33.
[84] Abdelmohsen K, Srikantan S, Kuwano Y, Gorospe M. miR-519 reduces cell proliferation by lowering RNA-binding protein HuR levels. Proc Natl Acad Sci U S A 2008;105:20297–302.
[85] Abdelmohsen K, Kim MM, Srikantan S, Mercken EM, Brennan SE, Wilson GM, et al. miR-519 suppresses tumor growth by reducing HuR levels. Cell Cycle 2010;9.
[86] Guo X, Wu Y, Hartley RS. MicroRNA-125a represses cell growth by targeting HuR in breast cancer. RNA Biol 2009;6:575–83.
[87] Yang L, Belaguli N, Berger DH. MicroRNA and colorectal cancer. World J Surg 2009;33:638–46.

[88] Tili E, Michaille JJ, Cimino A, Costinean S, Dumitru CD, Adair B, et al. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. J Immunol 2007;179:5082–9.
[89] Bazzoni F, Rossato M, Fabbri M, Gaudiosi D, Mirolo M, Mori L, et al. Induction and regulatory function of miR-9 in human monocytes and neutrophils exposed to proinflammatory signals. Proc Natl Acad Sci U S A 2009; 106:5282–7.
[90] Perry MM, Moschos SA, Williams AE, Shepherd NJ, Larner-Svensson HM, Lindsay MA. Rapid changes in microRNA-146a expression negatively regulate the IL-1beta-induced inflammatory response in human lung alveolar epithelial cells. J Immunol 2008;180:5689–98.
[91] Otto JC, DeWitt DL, Smith WL. N-glycosylation of prostaglandin endoperoxide synthases-1 and -2 and their orientations in the endoplasmic reticulum. J Biol Chem 1993;268:18234–42.
[92] Sevigny MB, Li CF, Alas M, Hughes-Fulford M. Glycosylation regulates turnover of cyclooxygenase-2. FEBS Lett 2006;580:6533–6.
[93] Mbonye UR, Yuan C, Harris CE, Sidhu RS, Song I, Arakawa T, et al. Two distinct pathways for cyclooxygenase-2 protein degradation. J Biol Chem 2008; 283:8611–23.
[94] Mbonye UR, Song I. Posttranscriptional and posttranslational determinants of cyclooxygenase expression. BMB Rep 2009;42:552–60.
[95] Tian J, Kim SF, Hester L, Snyder SH. S-nitrosylation/activation of COX-2 mediates NMDA neurotoxicity. Proc Natl Acad Sci U S A 2008;105:10537–40.
[96] Parfenova H, Balabanova L, Leffler CW. Posttranslational regulation of cyclooxygenase by tyrosine phosphorylation in cerebral endothelial cells. Am J Physiol 1998;274:C72–81.
[97] Kalgutkar AS, Crews BC, Rowlinson SW, Garner C, Seibert K, Marnett LJ. Aspirin-like molecules that covalently inactivate cyclooxygenase-2. Science 1998;280:1268–70.
[98] Jang BC, Sung SH, Park JG, Park JW, Bae JH, Shin DH, et al. Glucosamine hydrochloride specifically inhibits COX-2 by preventing COX-2 N-glycosylation and by increasing COX-2 protein turnover in a proteasome-dependent manner. J Biol Chem 2007;282:27622–3.
[99] Van Der Ouderaa FJ, Buytenhek M, Nugteren DH, Van Dorp DA. Acetylation of prostaglandin endoperoxide synthetase with acetylsalicylic acid. Eur J Biochem 1980;109:1–8.
[100] Groeger AL, Cipollina C, Cole MP, Woodcock SR, Bonacci G, Rudolph TK, et al. Cyclooxygenase-2 generates anti-inflammatory mediators from omega-3 fatty acids. Nat Chem Biol 2010;6:433–41.
[101] Chen C. COX-2s new role in inflammation. Nat Chem Biol 2010;6:401–2.
[102] Duvoix A, Delhalle S, Blasius R, Schnekenburger M, Morceau F, Fougere M, et al. Effect of chemopreventive agents on glutathione S-transferase P1-1 gene expression mechanisms via activating protein 1 and nuclear factor kappaB inhibition. Biochem Pharmacol 2004;68:1101–11.
[103] Folmer F, Blasius R, Morceau F, Tabudravu J, Dicato M, Jaspars M, et al. Inhibition of TNFalpha-induced activation of nuclear factor kappaB by kava (Piper methysticum) derivatives. Biochem Pharmacol 2006;71:1206–18.
[104] Schumacher M, Cerella C, Eifes S, Chateauvieux S, Morceau F, Jaspars M, et al. Heteronemin, a spongean sesterterpene, inhibits TNF alpha-induced NF-kappa B activation through proteasome inhibition and induces apoptotic cell death. Biochem Pharmacol 2010;79:610–22.
[105] Surh YJ, Chun KS, Cha HH, Han SS, Keum YS, Park KK, et al. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. Mutat Res 2001;480–481:243–68.
[106] Zhang F, Altorki NK, Mestre JR, Subbaramaiah K, Dannenberg AJ. Curcumin inhibits cyclooxygenase-2 transcription in bile acid- and phorbol ester-treated human gastrointestinal epithelial cells. Carcinogenesis 1999; 20:445–51.
[107] Huang MT, Lysz T, Ferraro T, Abidi TF, Laskin JD, Conney AH. Inhibitory effects of curcumin on in vitro lipoxygenase and cyclooxygenase activities in mouse epidermis. Cancer Res 1991;51:813–9.
[108] Chun KS, Keum YS, Han SS, Song YS, Kim SH, Surh YJ. Curcumin inhibits phorbol ester-induced expression of cyclooxygenase-2 in mouse skin through suppression of extracellular signal-regulated kinase activity and NF-kappaB activation. Carcinogenesis 2003;24:1515–24.
[109] Kang G, Kong PJ, Yuh YJ, Lim SY, Yim SV, Chun W, et al. Curcumin suppresses lipopolysaccharide-induced cyclooxygenase-2 expression by inhibiting activator protein 1 and nuclear factor kappab bindings in BV2 microglial cells. J Pharmacol Sci 2004;94:325–8.
[110] Sharma C, Kaur J, Shishodia S, Aggarwal BB, Ralhan R. Curcumin down regulates smokeless tobacco-induced NF-kappaB activation and COX-2 expression in human oral premalignant and cancer cells. Toxicology 2006; 228:1–15.
[111] Cho JW, Park K, Kweon GR, Jang BC, Baek WK, Suh MH, et al. Curcumin inhibits the expression of COX-2 in UVB-irradiated human keratinocytes (HaCaT) by inhibiting activation of AP-1: p38 MAP kinase and JNK as potential upstream targets. Exp Mol Med 2005;37:186–92.
[112] Camacho-Barquero L, Villegas I, Sanchez-Calvo JM, Talero E, Sanchez-Fidalgo S, Motilva V, et al. Curcumin, a Curcuma longa constituent, acts on MAPK p38 pathway modulating COX-2 and iNOS expression in chronic experimental colitis. Int Immunopharmacol 2007;7:333–42.
[113] Yu Z, Shah DM. Curcumin down-regulates Ets-1 and Bcl-2 expression in human endometrial carcinoma HEC-1-A cells. Gynecol Oncol 2007;106: 541–8.
[114] Tsatsanis C, Androulidaki A, Venihaki M, Margioris AN. Signalling networks regulating cyclooxygenase-2. Int J Biochem Cell Biol 2006;38:1654–61.
[115] Jang M, Pezzuto JM. Cancer chemopreventive activity of resveratrol. Drugs Exp Clin Res 1999;25:65–77.
[116] Subbaramaiah K, Chung WJ, Michaluart P, Telang N, Tanabe T, Inoue H, et al. Resveratrol inhibits cyclooxygenase-2 transcription and activity in phorbol ester-treated human mammary epithelial cells. J Biol Chem 1998; 273:21875–82.
[117] Park OJ, Surh YJ. Chemopreventive potential of epigallocatechin gallate and genistein: evidence from epidemiological and laboratory studies. Toxicol Lett 2004;150:43–56.
[118] Manna SK, Mukhopadhyay A, Aggarwal BB. Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. J Immunol 2000;164:6509–19.
[119] Lin YL, Lin JK. (-)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor-kappaB. Mol Pharmacol 1997; 52:465–72.
[120] Barthelman M, Bair 3rd WB, Stickland KK, Chen W, Timmermann BN, Valcic S, et al. (-)-Epigallocatechin-3-gallate inhibition of ultraviolet B-induced AP-1 activity. Carcinogenesis 1998;19:2201–4.
[121] Mutoh M, Takahashi M, Fukuda K, Matsushima-Hibiya Y, Mutoh H, Sugimura T, et al. Suppression of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells by chemopreventive agents with a resorcin-type structure. Carcinogenesis 2000;21:959–63.
[122] Liang YC, Huang YT, Tsai SH, Lin-Shiau SY, Chen CF, Lin JK. Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. Carcinogenesis 1999; 20:1945–52.
[123] Woo ER, Pokharel YR, Yang JW, Lee SY, Kang KW. Inhibition of nuclear factor-kappaB activation by 2',8''-biapigenin. Biol Pharm Bull 2006; 29:976–80.
[124] Srivastava JK, Pandey M, Gupta S. Chamomile: a novel and selective COX-2 inhibitor with anti-inflammatory activity. Life Sci 2009;85:663–9.
[125] Woo KJ, Jeong YJ, Inoue H, Park JW, Kwon TK. Chrysin suppresses lipopolysaccharide-induced cyclooxygenase-2 expression through the inhibition of nuclear factor for IL-6 (NF-IL6) DNA-binding activity. FEBS Lett 2005;579:705–11.
[126] Suh Y, Afaq F, Johnson JJ, Mukhtar H. A plant flavonoid fisetin induces apoptosis in colon cancer cells by inhibition of COX2 and Wnt/EGFR/NF-kappaB-signaling pathways. Carcinogenesis 2009;30:300–7.
[127] Moon DO, Kim MO, Kang SH, Choi YH, Kim GY. Sulforaphane suppresses TNF-alpha-mediated activation of NF-kappaB and induces apoptosis through activation of reactive oxygen species-dependent caspase-3. Cancer Lett 2009;274:132–42.
[128] Bhui K, Prasad S, George J, Shukla Y. Bromelain inhibits COX-2 expression by blocking the activation of MAPK regulated NF-kappa B against skin tumor-initiation triggering mitochondrial death pathway. Cancer Lett 2009; 282:167–76.
[129] Palozza P, Serini S, Maggiano N, Tringali G, Navarra P, Ranelletti FO, et al. beta-Carotene downregulates the steady-state and heregulin-alpha-induced COX-2 pathways in colon cancer cells. J Nutr 2005;135:129–36.
[130] Guo LY, Hung TM, Bae KH, Shin EM, Zhou HY, Hong YN, et al. Anti-inflammatory effects of schisandrin isolated from the fruit of Schisandra chinensis Baill. Eur J Pharmacol 2008;591:293–9.
[131] Corona G, Deiana M, Incani A, Vauzour D, Dessi MA, Spencer JP. Inhibition of p38/CREB phosphorylation and COX-2 expression by olive oil polyphenols underlies their anti-proliferative effects. Biochem Biophys Res Commun 2007;362:606–11.
[132] Tong X, Van Dross RT, Abu-Yousif A, Morrison AR, Pelling JC. Apigenin prevents UVB-induced cyclooxygenase 2 expression: coupled mRNA stabilization and translational inhibition. Mol Cell Biol 2007;27:283–96.
[133] Peng G, Dixon DA, Muga SJ, Smith TJ, Wargovich MJ. Green tea polyphenol (-)-epigallocatechin-3-gallate inhibits cyclooxygenase-2 expression in colon carcinogenesis. Mol Carcinog 2006;45:309–19.
[134] Annabi B, Currie JC, Moghrabi A, Beliveau R. Inhibition of HuR and MMP-9 expression in macrophage-differentiated HL-60 myeloid leukemia cells by green tea polyphenol EGCg. Leuk Res 2007;31:1277–84.
[135] Cao H, Kelly MA, Kari F, Dawson HD, Urban Jr JF, Coves S, et al. Green tea increases anti-inflammatory tristetraprolin and decreases pro-inflammatory tumor necrosis factor mRNA levels in rats. J Inflamm (Lond) 2007; 4:1.
[136] Tsang WP, Kwok TT. Epigallocatechin gallate up-regulation of miR-16 and induction of apoptosis in human cancer cells. J Nutr Biochem 2010; 21:140–6.
[137] Habbel P, Weylandt KH, Lichopoj K, Nowak J, Purschke M, Wang JD, et al. Docosahexaenoic acid suppresses arachidonic acid-induced proliferation of LS-174T human colon carcinoma cells. World J Gastroenterol 2009; 15:1079–84.
[138] Janakiram NB, Rao CV. Role of lipoxins and resolvins as anti-inflammatory and proresolving mediators in colon cancer. Curr Mol Med 2009;9: 565–79.
[139] Bommareddy A, Zhang X, Schrader D, Kaushik RS, Zeman D, Matthees DP, et al. Effects of dietary flaxseed on intestinal tumorigenesis in Apc(Min) mouse. Nutr Cancer 2009;61:276–83.

[140] Fradet V, Cheng I, Casey G, Witte JS. Dietary omega-3 fatty acids, cyclooxygenase-2 genetic variation, and aggressive prostate cancer risk. Clin Cancer Res 2009;15:2559–66.
[141] Lee CY, Sit WH, Fan ST, Man K, Jor IW, Wong LL, et al. The cell cycle effects of docosahexaenoic acid on human metastatic hepatocellular carcinoma proliferation. Int J Oncol 2010;36:991–8.
[142] Jin XJ, Kim EJ, Oh IK, Kim YK, Park CH, Chung JH. Prevention of UV-induced skin damages by 11,14,17-eicosatrienoic acid in hairless mice in vivo. J Korean Med Sci 2010;25:930–7.
[143] Lim K, Han C, Dai Y, Shen M, Wu T. Omega-3 polyunsaturated fatty acids inhibit hepatocellular carcinoma cell growth through blocking beta-catenin and cyclooxygenase-2. Mol Cancer Ther 2009;8:3046–55.
[144] Abramson SB. The role of COX-2 produced by cartilage in arthritis. Osteoarthritis Cartilage 1999;7:380–1.
[145] Hong H, Park YK, Choi MS, Ryu NH, Song DK, Suh SI, et al. Differential down-regulation of COX-2 and MMP-13 in human skin fibroblasts by glucosamine-hydrochloride. J Dermatol Sci 2009;56:43–50.