

Theme Issue Article

Transcription-based COX-2 inhibition: A therapeutic strategy

Kenneth K. Wu

Vascular Biology Research Center and Division of Hematology, Department of Medicine, The University of Texas Health Science Center at Houston, Houston, Texas, USA

Summary

Potent selective cyclooxygenase-2 (COX-2) inhibitors are effective in controlling inflammatory disorders but are associated with cardiovascular complications. Their clinical use has been severely limited. We propose that transcription-based inhibition of COX-2 expression represents a therapeutic strategy that may circumvent the undesired complications of COX-2 inhibitors. Reported data from several laboratories including ours have identified C/EBP β as a key transactivator mediating COX-2

transcriptional activation induced by diverse pro-inflammatory mediators. Results from our recent work show that sodium salicylate at pharmacological concentrations inhibits C/EBP β binding to COX-2 promoter by direct inhibition of p90 ribosomal S6 kinase (RSK). RSK phosphorylates C/EBP β and stimulates its binding to enhancer elements. We propose that RSK1/2 is a potential target for screening drugs with novel anti-inflammatory and anti-neoplastic therapeutic potentials.

Keywords

Cyclooxygenase-2, inflammation, C/EBP β , aspirin, P90 ribosomal S6 kinase

Thromb Haemost 2006; 96: 417–22

Introduction

Cyclooxygenase-2 (COX-2) is expressed at a very low level in most human cells at basal state and is highly inducible by diverse pro-inflammatory mediators including cytokines, lipopolysaccharide (LPS) and mitogenic factors (1). These factors induce robust COX-2 expression at the transcriptional level involving several key transactivators that bind to the promoter-enhancer region situated within 1 kb upstream of the transcription start site (2, 3). The induced COX-2 is primarily localized to the lumen of endoplasmic reticulum where it is coupled to phospholipase A₂ (PLA₂) and a downstream enzyme such as prostacyclin synthase (PGIS) (4). PLA₂ catalyzes the release of free arachidonic acid (AA) from membrane phospholipids. AA enters COX-2 where it is converted to PGG₂ by cyclooxygenase activity and PGG₂ is reduced to PGH₂ by peroxidase activity (5). PGH₂ is a common substrate for prostanooid production. For example, it is converted to PGE₂ by prostaglandin E synthase (PGES) and PGI₂ by PGIS. It has been suggested that COX-2 is co-induced with the microsomal (membrane-bound) PGES isoform in inflammatory cells which results in robust synthesis of PGE₂, an important pro-inflammatory mediator (6). In vascular endothelial cells, COX-2 is co-localized with PGIS which

results in abundant PGI₂ production (4). At physiological concentrations, PGI₂ plays an important role in maintaining vascular tone, vascular interaction with blood cells and protecting vascular cells from apoptosis (7, 8). There is strong evidence that COX-2/mPGES plays a major role in inflammatory joint disease and vascular inflammation (6). Selective COX-2 inhibitors such as rofecoxib and celecoxib prove to be effective in controlling joint inflammation and were widely used by a large population. Unfortunately, the coxibs inhibit not only the pro-inflammatory COX-2/PGES pathway in inflammatory cells but also the protective COX-2/PGIS pathway in vascular cells. It has been proposed that the cardiovascular complications now well documented among coxib users are due to inhibition of COX-2 derived PGI₂ by vascular endothelial cells (9–14). It should be noted that COX-2 derived PGI₂ plays important physiological roles in cells other than the vascular cells. It has been shown that COX-2 derived PGI₂ is crucial for in-vitro embryo development and uterine implantation (15, 16). COX-2 derived PGI₂ protects renal interstitial cells from apoptosis by hypertonicity and myocardial cells from damage by doxorubicin (17, 18). Long-term use of potent selective COX-2 inhibitors may therefore be associated with serious adverse effects besides the well recognized cardiovascular complications.

Kenneth K. Wu, MD
Vascular Biology Research Center and Division of Hematology
Department of Medicine
The University of Texas Health Science Center at Houston
6431 Fannin, MSB 5.016, Houston, Texas 77030, USA
Tel.: +1 713 500 6801, Fax: +1 713 500 6812
E-mail: Kenneth.K.Wu@uth.tmc.edu

Financial support:
This work is supported by grants from National Institutes of Health, USA
(P50 NS-23327 and R01 HL-50675).

Received July 18, 2006
Accepted after revision September 5, 2006

Prepublished online September 13, 2006 doi:10.1160/TH06-07-0403

Since transcriptional induction of COX-2 expression by pro-inflammatory mediators appears to depend on transactivators known to be involved in inflammation and tissue injury and the mechanisms by which the pro-inflammatory mediators induce binding of these transactivators to their respective binding sites are extensively elucidated, it may be feasible to develop therapeutic strategies centering on the inhibition of the DNA binding activity of the pro-inflammatory transactivators. An important aspect of this therapeutic approach is that COX-2 transcriptional activation by various pro-inflammatory mediators requires binding of multiple transactivators to the promoter and inhibition of the binding of a transactivator results in partial suppression of COX-2 expression. Importantly, it may be possible to design transcription-based control of COX-2 by targeting a biochemical process selectively used by pro-inflammatory mediators. Here, we will review the role of CCAAT/enhancer binding protein (C/EBP) in COX-2 transcription induced by common pro-inflammatory mediators and present evidence for the involvement of p90 ribosomal S6 kinase (RSK) in inducing C/EBP β binding to COX-2 promoter and activating COX-2 promoter function. We will also review the involvement of C/EBP in constitutive COX-2 overexpression in certain types of cancer cells.

Transcriptional activation of COX-2 by pro-inflammatory mediators

COX-2 promoters have been extensively characterized in murine and human cells (19–22). The promoter and enhancer regions of COX-2 from murine, bovine and human sources are similar with only minor differences. COX-2 promoters harbor a canonical TATA motif and several functionally important enhancer elements within ~500 bp upstream of the transcription start site (2, 3). A cyclic AMP response element (CRE) located at –53/–59 is absolutely essential for basal and induced COX-2 transcription as mutation of this site renders COX-2 promoter completely silent and unable to respond to exogenous stimuli (21, 24). Reported data have shown that IL-1 β , LPS, TNF α , or PMA stimulate COX-2 promoter activity by inducing binding of multiple transactivators to their respective binding sites, notably AP-1 site located close to CRE, C/EBP site (NF-IL6) located at –132/–124 and two κ B sites located at –213/–222 and –447/–438. A number of transactivators have been shown to be involved in COX-2 transcriptional activation by cytokines, LPS and growth factors. They include C-Jun/C-Fos (AP-1), p65/p50 NF- κ B, C/EBP β and C/EBP δ , NF-AT, SP-1, CREB-2/ATF2 (25–32). A pro-inflammatory mediator generally elicits the binding of a specific set of transactivators to the enhancer elements which in turn recruit 300/CBP co-activator (23). Among the transactivators, C/EBP β plays a crucial role in COX-2 expression in human and murine cells induced by cytokines, PMA, abnormal shear stress and LPS (26, 28, 33–36). C/EBP β belongs to the basic leucine zipper C/EBP family that comprises six members. C/EBP β shares sequence homology and functional properties with C/EBP δ and C/EBP α . C/EBP β has several truncated forms due to use of alternate translation codon ATG as a result of ribosomal translation

leakage (33, 38). A near full-length truncated form called LAP is transcriptionally active and is the main transactivator for COX-2, and a short form, LIP, is expressed and serves as a dominant negative form (39). PMA induces binding of full-length as well as LAP and LIP to the C/EBP site suggesting a dynamic regulation of C/EBP β transactivator activity by LIP (39). C/EBP δ constitutively binds C/EBP enhancer element. PMA treatment reduced C/EBP δ binding and replaced it with C/EBP β binding. Thus, C/EBP β binding to the COX-2 promoter is dynamically regulated. As will be described in more detail in the next sections, we consider C/EBP β as a potential target for transcription-based COX-2 suppression because of its common involvement in transactivating COX-2 promoter induced by pro-inflammatory mediators and its intrinsic dynamic regulation. Furthermore, its binding is activated by phosphorylation. By identifying the kinase(s) that phosphorylates and activates C/EBP β binding, it will be possible to use high throughput techniques to screen compounds that block C/EBP β -mediated COX-2 expression.

Roles of C/EBP β in constitutive COX-2 transcriptional activation in cancer cells

COX-2 is constitutively overexpressed in cancer cells (40–42). Its overexpression plays an important role in cancer growth and metastasis (43, 44). COX-2 promoter is constitutively activated in several types of cancer cells including colon and skin cancer cells. It has been shown that transfection of colon cancer cells (HCT-116) with a COX-2 promoter constructed into a luciferase expression vector results in constitutive expression of luciferase consistent with the presence in colon cancer cells of an active transactivation program (45). C/EBP and CRE enhancer elements in the COX-2 promoter/enhancer region were identified as being crucial for constitutive COX-2 transcriptional activation in colon cancer cells. The transactivators that bind these enhancer elements and activate COX-2 transcription have not been clearly defined. It has been reported that COX-2 transcription in colon cancer cells depends on ERK (46). As ERK and its downstream RSK are capable of phosphorylating and activating C/EBP β binding to COX-2 promoter, it is likely that C/EBP β plays a crucial role in COX-2 transcriptional activation in colon cancer cells.

COX-2 promoter has been shown to be constitutively activated in a skin cancer cell line (47). E-box and C/EBP enhancer elements have been shown to be essential for constitutive promoter activation. DNA-protein binding analysis by electrophoresis mobility shift assay shows binding of C/EBP β and C/EBP δ to the C/EBP site and E-box of COX-2 promoter (47). Taken together, these data suggest that C/EBP β binding to a specific C/EBP β enhancer element located close to the TATA box region plays an essential role in constitutive COX-2 transcriptional activation in colon and skin cancers and induced COX-2 transcriptional activation by pro-inflammatory mediators in normal inflammatory cells.

Salicylate inhibits COX-2 expression by targeting RSK-activated C/EBP β binding

The anti-inflammatory action of salicylate was recognized over a century ago, but the mechanism by which it controls inflammation remains to be elucidated. Besides being a natural product from diverse plants, salicylate is a key metabolite of aspirin *in vivo*. Salicylate is considered to contribute to the anti-inflammatory actions of aspirin. It is, therefore, of considerable importance to know how it works. Salicylate has very weak inhibitory action on COX-2 or COX-1 catalytic activity. Its anti-inflammatory action is unlikely due to blocking the COX-2 catalytic activity. It has been suggested that salicylate may exert its action by inhibiting DNA binding activity of transactivators that mediate the transcription of pro-inflammatory genes (48–50). Results from our laboratory indicate that salicylate at pharmacological concentrations (10^{-6} M to 10^{-4} M) inhibits COX-2 expression in human endothelial cells and fibroblasts induced by PMA, IL-1 β and LPS (51, 52). Salicylate inhibits COX-2 transcriptional activation by targeting C/EBP β transactivator. PMA, IL-1 β and LPS induce C/EBP β binding to a specific binding site (-132 to -124) on human COX-2 promoter. Inhibition of C/EBP β binding results in reduction of COX-2 protein levels by about 50% (51, 52). C/EBP β also plays a crucial role in transcriptional activation of inflammatory cytokines and other inflammatory genes (38). To test the hypothesis that salicylate is capable of inhibiting other C/EBP β -dependent genes, we investigated the effect of salicylate on inducible nitric oxide synthase (iNOS) expression in RAW 264.7, a murine macrophage. As COX-2 is also transcriptionally activated by LPS in this cell line, we also evaluated the effect of salicylate on COX-2 as a reference. The results show that salicylate inhibited LPS-induced iNOS and COX-2 expression at the transcriptional level through inhibition of C/EBP β binding to their binding sites on iNOS and COX-2 promoters, respectively. Surprisingly, salicylate inhibited iNOS transcription induced by LPS and interferon- γ (IFN γ) only at 4 hours (h) after stimulation but not at 8 h or 24 h (53). The reason for the time-dependent differential inhibition of iNOS transcription is unclear but might be due to complex LPS/IFN γ -induced signaling pathway changes at different time periods after stimulation.

C/EBP β at the resting state has weak DNA binding activity due to an intramolecular inhibitory element. Upon cellular activity, C/EBP β may be phosphorylated by a number of kinases (54–57). C/EBP β phosphorylation probably releases the intramolecular inhibition and exposes the DNA binding site. Our results have shown that PMA-induced C/EBP β binding is mediated by RSK. By using biochemical and molecular genetic approaches, we show that RSK-2 phosphorylates Thr-266 of C/EBP β (58). A dominant negative RSK-2 abrogates PMA-induced C/EBP β binding and PMA-induced COX-2 expression. To determine whether salicylate inhibits RSK activity, we transfected fibroblasts with a FLAG-tagged RSK. Following treatment with PMA for 4 h, cells were lysed and RSK activity was measured. Salicylate inhibited PMA-induced RSK activity. To determine whether salicylate directly inhibits RSK, cells were lysed and RSK was purified by FLAG-affinity column. The purified RSK was incubated with sodium salicylate at increasing concentrations.

Salicylate inhibited RSK activity in a concentration-dependent manner (58). These results indicate that salicylate has a direct inhibitory action on RSK1/2. As RSK is phosphorylated by ERK, and ERK has been shown to be capable of direct phosphorylation of C/EBP β , we determined whether salicylate inhibits ERK activity. The results show that salicylate had no effect on ERK. These results suggest that salicylate selectively inhibits RSK and thereby suppresses RSK-mediated C/EBP β binding. As RSK is involved in diverse pathophysiological processes including inflammation and diabetes (59, 60), and C/EBP β is a crucial transactivator for transcriptional activation of cytokines, iNOS and other pathophysiological important genes (38), salicylate may have a broad effect on diverse inflammatory disorders through its inhibition of RSK activity and C/EBP β binding.

An array of polyphenolic compounds purified from plants, fruits, tea and herbs has been shown to suppress COX-2 and iNOS transcriptional activation induced by pro-inflammatory mediators and mitogenic factors (61, 62). These compounds are active antioxidants as well. It is unclear how they suppress COX-2 expression. It would be interesting to test whether these purified compounds have a similar effect as salicylate on RSK activity and the consequent suppression of C/EBP β DNA binding. It has been suggested that some of the compounds may inhibit NF- κ B binding. Further studies are needed to elucidate the mechanisms by which the natural products inhibit COX-2 and iNOS. These studies will identify common targets that are valuable for transcription-based drug discovery.

Transcription-based COX-2 inhibition as a potential therapeutic strategy

As inflammation-mediated COX-2 transcriptional activation requires distinct transactivators such as C/EBP β , NF- κ B and C-Fos/C-Jun (AP-1), it is possible to design inhibitors to block selectively inflammation-coupled COX-2 expression. As illustrated in Figure 1, there are several potential therapeutic approaches such as suppressing the expression of C/EBP β by small interference RNA (siRNA) or blocking the activation of C/EBP β by targeting kinases that are responsible for inducing C/EBP β DNA binding. Our study suggests that RSK is a potential target for developing transcription-based therapy. RSK comprises a large family of proteins among which RSK-1 and RSK-2 (RSK1/2) share a high degree of sequence homology and overlapping biochemical and functional properties (63). Although RSK1/2 is a major downstream enzyme of p42/p44 mitogen-activated protein kinase (MAPK or ERK), it is activated by other upstream kinases such as protein kinase C (PKC) (64, 65). RSK1/2 phosphorylates serine or threonine residues of several transactivators including C-Fos (66, 67). RSK phosphorylates I κ B α and stimulates its degradation, thereby activating NF- κ B (68, 69). RSK1/2 has been implicated in diabetic myocardial ischemic injury (59) and prostate cancer cell proliferation (60). Our results have shown that RSK1/2 is pivotal in COX-2 transcription induced by pro-inflammatory mediators (58). Inhibition of RSK1/2 may not only block COX-2 transcriptional acti-

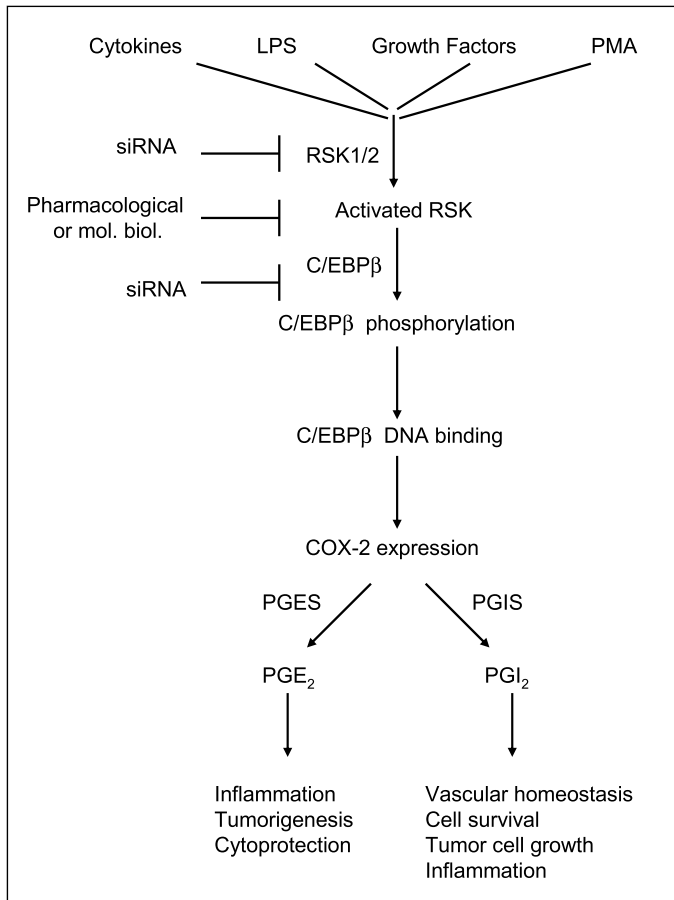


Figure 1: Schematic illustration of transcription-based COX-2 suppression. C/EBP β is one of the key transactivators for mediating COX-2 transcription. It is phosphorylated by several kinases. Pro-inflammatory mediators activate ribosomal S6 kinase 1/2 (RSK) which enhances C/EBP β binding to COX-2 promoter and upregulates COX-2 expression. Elevated COX-2 generates a high level of PGE₂ and PGI₂ by coupling with PGE synthase (PGES) and PGI synthase (PGIS) respectively. PGE₂ is considered to be a key mediator of inflammation and tumorigenesis but may play a role in cytoprotection and reproduction. PGI₂ is generally considered to protect blood vessels but may be involved in colon cancer growth. We propose that inhibition of RSK or C/EBP β expression by small interference (si) RNA or other molecular biological (mol. biol.) means and pharmacological or molecular genetic inhibition of RSK activity may have therapeutic potential as these approaches reduce COX-2 to a level sufficient for controlling inflammation or tumorigenesis without jeopardizing the protective and physiological function of COX-2. LPS denotes lipopolysaccharide and PMA phorbol 12-myristate 13-acetate.

vation but may also block activation of other pro-inflammatory genes. Thus, with respect to transcriptional control of inflammatory genes and suppression of pathophysiological processes such as tissue inflammation and injury, RSK1/2 appears to be a highly suitable therapeutic target. Since salicylate inhibits RSK1/2, it may be predicted that an array of small-molecular-weight compounds bearing the core structure of salicylate will be identified by high throughput RSK screening. These compounds will have therapeutic potential for a wide variety of human diseases.

C/EBP β appears to be an excellent target for drug devel-

opment. C/EBP β is essential for COX-2 transcription induced by diverse pro-inflammatory mediators. Furthermore, it plays a key role in mediating constitutive overexpression in cancer cells. There are several C/EBP β isoforms which are derived from alternate translation start site due to ribosomal leakage (70). Functionally, the LAP isoform mediates C/EBP β transactivation activity while LIP, a short isoform, blocks the transactivation activity of LAP (39, 70). There may be several approaches to control C/EBP β binding activity. As has been described above, one approach is to inhibit kinase that phosphorylates and induces C/EBP β DNA binding. In addition to inhibiting RSK1/2 activity, targeting other kinases such as CaMK IV may have therapeutic value. Another approach is to develop agents that directly inhibit C/EBP β binding. One candidate is the dominant-negative LIP. LIP is capable of binding C/EBP β (LAP) and C/EBP δ and preventing DNA binding of these C/EBP isoforms.

NF- κ B plays an essential role in COX-2 and iNOS transcriptional activation induced by diverse pro-inflammatory mediators, notably tumor necrosis factor- α (TNF- α) (22). NF- κ B comprises five isoforms, two in the NF- κ B family (NF- κ B1 also known as p105/p50 and NF- κ B, known as p100/p52) and three in the Rel family (C-Rel or p75, RelA or p65 and RelB or p68). These isoforms form homodimers or heterodimers (71). All isoforms are sequestered in cytosol by binding to an inhibitor known as I κ B (72). Upon activation, I κ B is phosphorylated by a number of kinases including I κ B kinases and RSK1/2 which facilitates I κ B ubiquitination and degradation (68, 69). Free NF- κ B translocates to the nucleus and binds to specific promoter enhancer elements. To suppress NF- κ B transactivation actions, one target is the kinase(s) that phosphorylates I κ B. Compounds to inhibit NF- κ B are being developed. Other important transcription targets include AP-1, which is a heterodimer of C-Fos and C-Jun family proteins. As the approaches are in principle similar to these for C/EBP β and NF- κ B, they will not be described in detail here.

One caveat of the transcription-based COX-2 inhibition is the lack of specificity. For example, RSK1/2 is involved in phosphorylating diverse proteins, some of which may exert beneficial physiological functions such as cell survival (67). Inhibition of RSK may therefore shift the balance between pro- and antiapoptotic factors in cells.

Conclusion and perspective

Transcription-based COX-2 inhibition represents a useful therapeutic strategy to treat COX-2 mediated inflammatory disorders and tissue injury. As COX-2 transcription is coupled to pro-inflammatory stimuli, it is possible to selectively block inflammation-stimulated COX-2 expression without suppressing the physiological function of COX-2. Our results have shown that RSK1/2 induces C/EBP β binding, thereby activating COX-2 transcription, and salicylate suppresses C/EBP β -mediated COX-2 expression by inhibiting RSK1/2. Based on these results, we propose that RSK1/2 is a target for drug screening and discovery. Future plans include developing high throughput techniques for screening small-molecular-weight compounds and selecting suitable compounds for animal and human studies.

References

1. Wu KK. Inducible cyclooxygenase and nitric oxide synthase. *Adv Pharmacol* 1995; 33: 179–207.
2. Fletcher BS, Kujubu DA, Perrin DM, et al. Structure of the mitogen-inducible TIS10 gene and demonstration that the TIS10-encoded protein is a functional prostaglandin G/H synthase. *J Biol Chem* 1992; 267: 4338–44.
3. Tazawa R, Xu XM, Wu KK, et al. Characterization of the genomic structure, chromosomal location and promoter of human prostaglandin H synthase-2 gene. *Biochem Biophys Res Commun* 1994; 203: 190–9.
4. Liou JY, Shyue SK, Tsai MJ, et al. Colocalization of prostacyclin synthase with prostaglandin H synthase-1 (PGHS-1) but not phorbol ester-induced PGHS-2 in cultured endothelial cells. *J Biol Chem* 2000; 275: 15314–20.
5. Smith WL, Garavito RM, DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. *J Biol Chem* 1996; 271: 33157–60.
6. Cipollone F, Prontera C, Pini B, et al. Overexpression of functionally coupled cyclooxygenase-2 and prostaglandin E synthase in symptomatic atherosclerotic plaques as a basis of prostaglandin E(2)-dependent plaque instability. *Circulation* 2001; 104: 921–7.
7. Moncada S, Vane JR. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A₂, and prostacyclin. *Pharmacol Rev* 1978; 30: 293–331.
8. Liou JY, Lee S, Ghelani D, et al. Protection of Endothelial Survival by Peroxisome Proliferator-Activated Receptor- δ Mediated 14–3–3 Upregulation. *Arterioscler Thromb Vasc Biol* 2006 (in press).
9. FitzGerald GA. Coxibs and cardiovascular disease. *N Engl J Med* 2004; 351: 1709–11.
10. Mukherjee D, Nissen SE, Topol EJ. Risk of cardiovascular events associated with selective COX-2 inhibitors. *J Am Med Assoc* 2001; 286: 954–9.
11. McAdam BF, Catella-Lawson F, Mardini IA, et al. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. *Proc Natl Acad Sci USA* 1999; 96: 272–7.
12. FitzGerald GA, Smith B, Pedersen AK, et al. Increased prostacyclin biosynthesis in patients with severe atherosclerosis and platelet activation. *N Engl J Med* 1984; 310: 1065–8.
13. Belton O, Byrne D, Kearney D, et al. Cyclooxygenase-1 and -2-dependent prostacyclin formation in patients with atherosclerosis. *Circulation* 2000; 102: 840–5.
14. Cheng Y, Austin SC, Rocca B, et al. Role of prostacyclin in the cardiovascular response to thromboxane A₂. *Science* 2002; 296: 539–41.
15. Lim H, Gupta RA, Ma WG, et al. Cyclooxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPAR δ . *Genes Dev* 1999; 13: 1561–74.
16. Huang JC, Wun WS, Goldsby JS, et al. Cyclooxygenase-2-derived endogenous prostacyclin enhances mouse embryo hatching. *Hum Reprod* 2004; 19: 2900–6.
17. Hao CM, Komhoff M, Guan Y, et al. Selective targeting of cyclooxygenase-2 reveals its role in renal medullary interstitial cell survival. *Am J Physiol* 1999; 277: F352–F359.
18. Adnerley SR, Fitzgerald DJ. Oxidative damage of cardiomyocytes is limited by extracellular regulated kinases -mediated induction of cyclooxygenase-2. *J Biol Chem* 1999; 274: 5038–46.
19. Herschman HR. Prostaglandin synthase 2. *Biochim Biophys Acta* 1996; 1299: 125–40.
20. Wadleigh DJ, Reddy ST, Kopp E, et al. Transcriptional activation of the cyclooxygenase-2 gene in endotoxin-treated RAW 264.7 macrophages. *J Biol Chem* 2000; 275: 6259–66.
21. Schroer K, Zhu Y, Saunders MA, et al. Obligatory role of cyclic adenosine monophosphate response element in cyclooxygenase-2 promoter induction and feedback regulation by inflammatory mediators. *Circulation* 2002; 105: 2760–5.
22. Deng WG, Zhu Y, Wu KK. Up-regulation of p300 binding and p50 acetylation in tumor necrosis factor- α -induced cyclooxygenase-2 promoter activation. *J Biol Chem* 2003; 278: 4770–7.
23. Deng WG, Zhu Y, Wu KK. Role of p300 and PCAF in regulating cyclooxygenase-2 promoter activation by inflammatory mediators. *Blood* 2004; 103: 2135–42.
24. Inoue H, Nanayama T, Hara S, et al. The cyclic AMP response element plays an essential role in the expression of the human prostaglandin-endoperoxide synthase 2 gene in differentiated U937 monocytic cells. *FEBS Lett* 1994; 15: 51–4.
25. Hernandez GL, Volpert OV, Iniguez MA, et al. Selective inhibition of vascular endothelial growth factor-mediated angiogenesis by cyclosporin A: roles of the nuclear factor of activated T cells and cyclooxygenase 2. *J Exp Med* 2001; 193: 607–20.
26. Thomas B, Berenbaum F, Humbert L, et al. Critical role of C/EBP δ and C/EBP β factors in the stimulation of the cyclooxygenase-2 gene transcription by interleukin-1 β in articular chondrocytes. *Eur J Biochem* 2000; 267: 6798–809.
27. Mestre JR, Mackrell PJ, Rivadeneira DE, et al. Redundancy in the signaling pathways and promoter elements regulating cyclooxygenase-2 gene expression in endotoxin-treated macrophage/monocytic cells. *J Biol Chem* 2001; 276: 3977–82.
28. Caivano M, Gorgoni B, Cohen P, et al. The induction of cyclooxygenase-2 mRNA in macrophages is biphasic and requires both CCAAT enhancer-binding protein beta (C/EBP beta) and C/EBP delta transcription factors. *J Biol Chem* 2001; 276: 48693–701.
29. Eliopoulos AG, Dumitru CD, Wang CC, et al. Induction of COX-2 by LPS in macrophages is regulated by Tpl2-dependent CREB activation signals. *EMBO J* 2002; 21: 4831–40.
30. Reddy ST, Wadleigh DJ, Herschman HR. Transcriptional regulation of the cyclooxygenase-2 gene in activated mast cells. *J Biol Chem* 2000; 275: 3107–13.
31. Yamamoto K, Arakawa T, Ueda N, et al. Transcriptional roles of nuclear factor kappa B and nuclear factor-interleukin-6 in the tumor necrosis factor alpha-dependent induction of cyclooxygenase-2 in MC3T3-E1 cells. *J Biol Chem* 1995; 270: 31315–20.
32. Inoue H, Yokoyama C, Hara S, et al. Transcriptional regulation of human prostaglandin-endoperoxide synthase-2 gene by lipopolysaccharide and phorbol ester in vascular endothelial cells. *J Biol Chem* 1995; 270: 24965–71.
33. Wu KK, Liou JY, Cieslik K. Transcriptional Control of COX-2 via C/EBP β . *Arterioscler Thromb Vasc Biol* 2005; 25: 679–85.
34. Chen J, Zhao M, Rao R, et al. C/EBP β and its binding element are required for NF κ B-induced COX2 expression following hypertensive stress. *J Biol Chem* 2005; 280: 16354–9.
35. Sorli CH, Zhangdagger H-J, Armstrongdagger MB, et al. Basal expression of cyclooxygenase-2 and nuclear factor-interleukin 6 are dominant and coordinately regulated by interleukin 1 in the pancreatic islet. *Proc Natl* 1998; 95: 1788–93.
36. Ogasawara A, Arakawa T, Kaneda T, et al. Fluid shear stress-induced cyclooxygenase-2 expression is mediated by C/EBP beta, cAMP-response element-binding protein, and AP-1 in osteoblastic MC3T3-E1 cells. *J Biol Chem* 2001; 276: 7048–54.
37. Wedel A, Ziegler-Heitbrock HW. The C/EBP family of transcription factors. *Immunobiology* 1995; 193: 171–85.
38. Akira S, Kishimoto TNF. IL6 and NF-kappa B in cytokine gene regulation. *Adv Immunol* 1997; 65: 1–46.
39. Zhu Y, Saunders MA, Yeh H, et al. Dynamic regulation of cyclooxygenase-2 promoter activity by isoforms of CCAAT/enhancer-binding proteins. *J Biol Chem* 2002; 277: 6923–8.
40. Eberhart CE, Coffey RJ, Radhika A, et al. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994; 107: 1183–8.
41. Kargman SL, O'Neill GP, Vickers PJ, et al. Expression of cyclooxygenase G/H synthase-1 and -2 protein in human colon cancer. *Cancer Res* 1995; 55: 2556–9.
42. Sano H, Kawahito Y, Wilder RL, et al. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. *Cancer Res* 1995; 55: 3785–9.
43. Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 1995; 83: 493–501.
44. Tsujii M, Kawano S, DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci USA* 1997; 94: 3336–40.
45. Kutcher W, Jones DA, Matsunami N, et al. Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: evidence for a transcriptional effect. *Proc Natl Acad Sci USA* 1996; 93: 4816–20.
46. Shao J, Sheng H, Inoue H, et al. Regulation of constitutive cyclooxygenase-2 expression in colon carcinoma cells. *J Biol Chem* 2000; 275: 33951–6.
47. Kim Y, Fischer SM. Transcriptional regulation of cyclooxygenase-2 in mouse skin carcinoma cells. *J Biol Chem* 1998; 273: 27686–94.
48. Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. *Science* 1994; 265: 956–9.
49. Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. *Nature* 1998; 396: 7–80.
50. Frantz B, O'Neill EA. The effect of sodium salicylate and aspirin on NF-kappa B. *Science* 1995; 270: 2017–9.
51. Xu XM, Sansores-Garcia L, Chen XM, et al. Suppression of inducible cyclooxygenase 2 gene transcription by aspirin and sodium salicylate. *Proc Natl Acad Sci USA* 1999; 96: 5292–7.
52. Saunders MA, Sansores-Garcia L, Gilroy DW, et al. Selective suppression of CCAAT/enhancer-binding protein beta binding and cyclooxygenase-2 promoter activity by sodium salicylate in quiescent human fibroblasts. *J Biol Chem* 2001; 276: 18897–904.
53. Cieslik K, Zhu Y, Wu KK. Salicylate suppresses macrophage NOS-2 and COX-2 expression by inhibiting C/EBP β binding via a common signaling pathway. *J Biol Chem* 2002; 277: 49304–10.
54. Nakajima T, Kinoshita S, Sasagawa T, et al. Phosphorylation at threonine-235 by a ras-dependent mitogen-activated protein kinase cascade is essential for transcription factor NF-IL6. *Proc Natl Acad Sci USA* 1993; 90: 2207–11.
55. Trautwein C, Caelles C, van der Geer P, et al. Transactivation by NF-IL6/LAP is enhanced by phosphorylation of its activation domain. *Nature* 1993; 364: 544–7.

56. Buck M, Poli V, van der Geer P, et al. Phosphorylation of rat serine 105 or mouse threonine 217 in C/EBP beta is required for hepatocyte proliferation induced by TGF alpha. *Mol Cell* 1999; 4: 1087-92.
57. Wegner M, Cao Z, Rosenfeld MG. Calcium-regulated phosphorylation within the leucine zipper of C/EBP beta. *Science* 1992; 256: 370-3.
58. Cieslik KA, Zhu Y, Shtivelband M, et al. Inhibition of p90 ribosomal S6 kinase-mediated CCAAT/enhancer-binding protein beta activation and cyclooxygenase-2 expression by salicylate. *J Biol Chem* 2005; 280: 18411-7.
59. Itoh S, Ding B, Shishido T, et al. Role of p90 ribosomal S6 kinase-mediated prorenin-converting enzyme in ischemic and diabetic myocardium. *Circulation* 2006; 113: 1787-98.
60. Clark DE, Errington TM, Smith JA, et al. The serine/threonine protein kinase, p90 ribosomal S6 kinase, is an important regulator of prostate cancer cell proliferation. *Cancer Res* 2005; 65: 3108-16.
61. Subbaramaiah K, Chung WJ, Michaluart P, et al. Resveratrol inhibits cyclooxygenase-2 transcription and activity in phorbol ester-treated human mammary epithelial cells. *J Biol Chem* 1998; 273: 21875-82.
62. Liang YC, Huang YT, Tsai SH, et al. Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. *Carcinogenesis* 1999; 20: 1945-52.
63. Shimamura A, Ballif BA, Richards SA, et al. Rsk1 mediates a MEK-MAP kinase cell survival signal. *J Curr Biol* 2000; 10: 127-35.
64. Abe J, Okuda M, Huang Q, et al. Reactive oxygen species activate p90 ribosomal S6 kinase via Fyn and Ras. *J Biol Chem* 2000; 275: 1739-48.
65. Itoh S, Ding B, Bains CP, et al. Role of p90 ribosomal S6 kinase (p90RSK) in reactive oxygen species and protein kinase C beta (PKC-beta)-mediated cardiac troponin I phosphorylation. *J Biol Chem* 2005; 280: 24135-42.
66. Chen RH, Chung J, Blenis J. Regulation of pp90rsk phosphorylation and S6 phosphotransferase activity in Swiss 3T3 cells by growth factor-, phorbol ester-, and cyclic AMP-mediated signal transduction. *Mol Cell Biol* 1991; 11: 1861-7.
67. Xing J, Ginty DD, Greenberg ME. Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. *Science* 1996; 273: 959-63.
68. Ghoda L, Lin X, Greene WC. The 90-kDa ribosomal S6 kinase (pp90rsk) phosphorylates the N-terminal regulatory domain of I kappa B alpha and stimulates its degradation *in vitro*. *J Biol Chem* 1997; 272: 21281-8.
69. Schouten GJ, Vertegaal AC, Whiteside ST, et al. I kappa B alpha is a target for the mitogen-activated 90 kDa ribosomal S6 kinase. *EMBO J* 1997; 16: 3133-44.
70. Descombes P, Schibler UA. liver-enriched transcriptional activator protein, LAP, and a transcriptional inhibitory protein, LIP, are translated from the same mRNA. *Cell* 1991; 67: 569-79.
71. Dixit V. NF-kB Signaling: Many roads lead to Madrid. *Cell* 2002; 111: 615-9.
72. Karin M. The beginning of the end: I kappa B kinase (IKK) and NF-kappa B activation. *J Biol Chem* 1999; 274: 27339-42.