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MINI REVIEW

Cross-talks between cyclooxygenase-2 and tumor suppressor protein p53: Balancing life and death during inflammatory stress and carcinogenesis

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Overexpression of Cyclooxygenase-2 (COX-2) is observed in most tumor types. Increased COX-2 activity and synthesis of prostaglandins stimulates proliferation, angiogenesis, invasiveness and inhibits apoptosis. Many stress and proinflammatory signals induce COX-2 expression, including oxyradicals or DNA-damaging agents. The latter also induces p53, a transcription factor often inactivated by mutation in cancer. Several studies have identified complex cross-talks between p53 and COX-2, whereby p53 can either up- or down-regulate COX-2, which in turn controls p53 transcriptional activity. However, the molecular basis of these effects are open to debate, in particular since no p53 binding sequences have been identified in COX-2 regulatory regions. In this review, we summarize the molecular mechanisms by which COX-2 contributes to carcinogenesis and discuss the experimental set-up, results and conclusions of studies analyzing cross-talks between p53 and COX-2. We propose 2 scenarios accounting for overexpression of COX-2 in precursor and cancer lesions. In the "inflammatory" scenario, p53, activated by DNA damage induced by oxygen and nitrogen species, recruits NF-kappaB to activate COX-2, resulting in antiapoptotic effects that contribute to cell expansion in inflammatory precursor lesions. In the "constitutive proliferation" scenario, oncogenic stress due to activation of growth signaling cascades may upregulate COX-2 promoter independently of NF-kappaB and p53, synergizing with TP53 mutation to promote cancer progression. These 2 scenarios, although not mutually exclusive, may account for the diversity of the correlations between COX-2 expression and TP53 mutation, which vary according to cancer types and biological contexts, and have implications for the use of COX-2 inhibitors in cancer prevention and therapy.

Key words: p53; COX-2; cancer; inflammation; apoptosis; DNA damage; COX-2 inhibitors

Cyclooxygenases (COX) are heme-containing glycoproteins catalyzing the conversion of arachidonic acid to precursors of prostaglandins, prostacyclins and thromboxanes, which are inhibited by nonsteroidal antiinflammatory drugs (NSAIDs). Two COX enzymes have been described. COX-1 is constitutively expressed in most tissues and is involved in tissue homeostasis. COX-2 is expressed only under certain extracellular or intracellular stimuli, including mitogens, tumor promoters, cytokines, hormones and infectious agents, and is a component of cellular responses to inflammation[1,2] (Fig. 1). Induction of COX-2 is transient, with return to baseline levels within 24–48 hr following induction. The sequence identified as COX-3 consists into a splice variant of COX-1 that retains intron 1, resulting in a frameshift in the RNA message. This sequence does not give rise to an active cyclooxygenase protein.[3] The roles of COX-1 and COX-3 will not be further discussed in this review.

COX-2 is overexpressed in many solid tumors, including colon, prostate, breast, esophagus, lung, bladder and pancreas (Table I). These neoplastic tissues often contain high concentrations of prostaglandins. A significant inverse relationship between COX-2 overexpression and survival of cancer patients has been reported in retrospective studies.[25,35] In general, COX-2 expression is higher in well-to-moderately differentiated tumors and in metastasis than in low-differentiated tumors. For example, upregulation of COX-2 has been detected in up to 90% of colorectal carcinoma and 40% of adenomas, but not in hyperplastic polyps or in normal colorectal mucosa.[36] In lung and esophageal adenocarcinomas, increased COX-2 expression is detectable in precursor lesions of such as atypical adenomatous hyperplasia and Barrett's esophagus, respectively.[37,38] Overall, overexpression of COX-2 is associated with tumor aggressiveness and adverse patient outcome, identifying COX-2 as an independent indicator of poor prognostic.[39]

Many signals that activate COX-2 also induce the tumor suppressor p53, a transcription factor that accumulates after post-translation modification in response to DNA damage (Fig. 1). Induction of p53 results in a complex network of antiproliferative responses such as cell cycle arrest, DNA repair or apoptosis (reviewed in Ref. 40). There is controversy on whether p53 induction and upregulation of COX-2 cooperate or antagonize with each other before or during the onset of cancer.[16,41,41] In this review, we present an overview of cross-talks between p53 and COX-2, and we discuss the pathological contexts in which these cross-talks may take place.

Roles of COX 2 in carcinogenesis

Lessons from animal models

Different groups have reported results on transgenic mice over-expressing COX-2 under the control of tissue-specific promoters (Table II). In mouse skin, over-expression of COX-2 in basal keratinocytes resulted in high levels of prostaglandins, with skin differentiation defects and hyperplastic features at discrete sites. Mice do not spontaneously develop skin tumors but do so after a single application of 7,12-dimethylbenz[a]anthracene (DMBA).[48] This observation suggests that over-expression of COX-2 transforms epidermis into an "autopromoted" state with sensitization to genotoxic agents. In another mouse model, aberrant expression of COX-2 under the control of keratin 5-promoter, resulted into pancreatic intraepithelial neoplasia, without any carcinogen administration.[46] In the mammary gland, involution is delayed in COX-2 transgenic mice, with a decrease in apoptotic index. Multiparous but not virgin females show an increased incidence of focal mammary gland hyperplasia, dysplasia and transformation into metastatic tumors.[45]

In COX-2 null mice, several physiopathological defects result in reduced viability, including renal dysplasia, cardiac fibrosis,

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peritonitis and accompanying lesions of the abdominal cavity. Female COX-2−/− mice are infertile. COX-2 deficiency failed to alter inflammatory responses in several standard models, but reduction of endotoxin-induced hepatocellular cytotoxicity was observed.[50,51] This phenotype appears to mitigate after a few generations, suggesting that mice adapt to the lack of COX-2. In hybrids between COX-2 deficient mice and $APC^{\Delta 716}$ knockout mice, a model of human familial adenomatous polyposis (FAP), loss of COX-2 activity reduces the number and size of intestinal polyps that spontaneously form as a consequence of APC mutation (Table II). Furthermore, treating $APC^{\Delta 716}$ mice with rofecoxib, a specific COX-2 inhibitor, reduces polyp numbers.[43] Additional evidence shows that genetic disruption of the prostaglandin E receptor EP1 reduces the formation of aberrant crypt foci induced by azoxymethane.[52] Together, the results on transgenic COX-2 and COX-2 null mice provide evidence for a key role of COX-2 in promoting tumourigenesis.

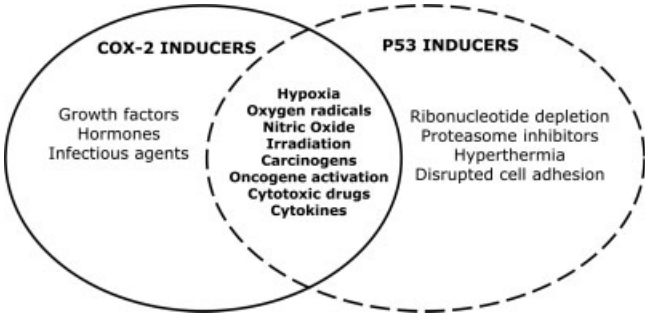

FIGURE 1 – Venn diagram showing COX-2 and p53 inducers. The overlapping area between circles indicates agents capable of inducing both COX-2 and p53. Whether induction proceeds through independent mechanisms or involves cross-talks between p53 and NF-kappaB is further discussed in this review.

Cellular mechanisms

Many studies using COX-2 inhibitors show reduction of cell growth in tumor cell lines and *in vivo* tumor models, including colon, skin epidermal, hepatic, esophageal and lung cancers.[48,53–55] In colon cancer cell lines, combined inhibition of COX-2 and EGF-like HER-2/neu pathways synergistically reduces cell growth.[56] Overexpression of COX-2 in mammary cells has been shown to increase the activity of Cytochrome P450 (CYP) aromatase (aromatase), a product of the CYP19 gene that catalyzes the synthesis of estrogens from androgens.[57] Overexpression of HER-2/neu is also associated with increased aromatase activity, suggesting that COX-2 may act as a functional intermediate linking HER-2/neu and aromatase and that inhibitors of PGE_2 synthesis may contribute to suppress estrogen biosynthesis in breast tissue.[58]

The increased tumorigenic potential of cells overexpressing COX-2 is thought to be due to resistance to or escape from apoptosis. COX-2 may prevent apoptosis by several mechanisms, including (*i*) synthesizing prostaglandins acting as antiapoptotic, survival factors[59]; (*ii*) metabolizing arachidonic acid, a proapoptotic substrate that activates caspase-3 and modulates mitochondrial permeability[60]; (*iii*) increasing the expression of the antiapoptotic protein Bcl-2[45,59] and (*iv*) activating the serine threonine kinase Akt.[61] Conversely, COX-2 inhibitors are able to induce expression of the pro-apoptotic factors Gadd153[62] and Fas,[63] to sensitize human colon carcinoma cells to the tumor necrosis factor-related

TABLE I – SUMMARY OF STUDIES ON COX-2 OVEREXPRESSION IN SOLID TUMORS AND CONCORDANCE WITH p53 EXPRESSION OR MUTATION STATUS

Tumor site	Percentage of COX-2 overexpression	p53 correlation	Reference
Breast	66.9%	Inverse correlation between COX-2 and p53 expression	4
Breast	34.7%	Correlation between COX-2 and p53 expression	5
Breast	32.3%	Correlation between COX-2 and p53 expression	6
Bladder	100%	NA	7
Bladder	66%	NA	8
Cervix	43%	NA	9
Cervix	30%	NA	10
Colorectum	NA	No association with p53 expression	11
Colorectum	65%	Correlation between COX-2 and p53 expression	12
Colorectum	73%	NA	13
Endometer	39%	NA	14
Endometer	50%	No association with p53 expression	15
Oesophagus	SCC: 11% ADC: 44%	SCC: No association with TP53 status ADC: Correlation between wild-typeTP53 and COX-2 expression	16
Oesophagus	SCC: 74%	Correlation between mutant TP53 and COX-2 expression	17
Oesophagus	SCC: 74%	Correlation between COX-2 and p53 expression	18
Oesophagus	SCC: 69%	No association with TP53 status	19
Oesophagus	SCC: 91% ADC: 78%	NA	20
Gall bladder	ADC: 59.2%	Correlation between COX-2 and p53 expression	21
Head and neck	SCC: 100%	NA	22
Liver	HCC: 97%	NA	23
Lung	ADC: 90%	NA	24
Lung	ADC: 72%	NA	25
Lung	SCC: 57.4% ADC: 74.5%	No association with TP53 status	26
Oral cavity and pharynx	SCC: 88%	No association with p53 expression	27
Ovary	45%	NA	28
Pancreas	66%	Correlation between COX-2 and p53 expression	29
Pancreas	77%	NA	30
Prostate	83%	NA	31
Skin	SCC: 50% BCC: 80%	No association with p53 expression	32
Stomach	48.7%	Correlation between mutant TP53 and COX-2 expression	33
Stomach	100%	NA	34

ADC, adenocarcinoma; BCC, basal cell carcinoma; HCC, hepatocellular carcinoma; SCC, squamous cell carcinoma; NA, not available in the publication.

TABLE 2 – TUMOR PHENOTYPES IN COX-2 GENETICALLY MODIFIED MICE

Mouse model	Genetic alteration	Tumourigenesis	Reference
Intestine	COX-2 knockout/ APC knockout	Reduced number of colon polyps and adenoma formation	43
Mammary gland	HER2/neu transgenic/ COX-2 knockout	HER/neu-induced multiple focal tumors and mammary hyperplasia were reduced in *COX-2* knockout mice. Decreased vascularization was observed in *COX-2*-null mammary glands	44
Mammary gland	COX-2 transgenic	Multiparous females exhibited focal mammary gland hyperplasia, dysplasia and metastasis	45
Pancreas	COX-2 transgenic	Development of intraepithelial neoplasms	46
Skin	COX-2 transgenic	Development of epidermal hyperplasia	47
Skin	COX-2 transgenic	Tumor formation just after DMBA administration	48
Urinary bladder	COX-2 transgenic	Development of transitional cell hyperplasia and carcinoma	49

apoptosis-inducing ligand (TRAIL),[64] and to decrease survivin levels.[65] Survivin inhibits apoptosis *via* its BIR (baculovirus inhibitor of apoptosis repeat) protein domain by either directly or indirectly interfering with the function of caspases 3 and 7.[65,66]

Extracellular mechanisms

Increased COX-2 activity and enhanced production of prostaglandins modulates angiogenesis, cell adhesion and metastasis. Overexpression of COX-2 promotes synthesis of VEGF. In a transmembrane experimental system, increased expression of COX-2 in colon cancer cells promotes mobility and vascular morphogenesis by cocultured endothelial cells.[67] These effects are reversed by COX-2 inhibitors. Angiogenic effects of COX-2 are mediated through products of arachidonic acid metabolism and involve multiple effectors in addition to increased VEGF (reviewed in Ref. 68). A role of COX-2 expression in the promotion of tumor invasiveness has been proposed based on its capacity to increase the expression of different matrix metalloproteinases and of CD44, the cell surface receptor of hyaluronate.[69]

Roles of p53 in regulation of COX-2 expression and activity

p53 in cancer and inflammation

The tumor suppressor p53 is activated by a wide range of physio-pathological stimuli, including various types of DNA damaging stress, agents and factors that stall DNA replication, hypoxia, ribonucleotide depletion, perturbation of cell adhesion and excessive or untimely mitogenic signals (Fig. 1).[70] Induction and nuclear accumulation of p53 results in the transcriptional regulation of genes collectively involved in cell cycle arrest, DNA repair, apoptosis, differentiation and some aspects of development. The consequences of p53 induction depend upon the nature and intensity of the signals, the cell type, and the cell particular history. While stem cells with high proliferative potential tend to undergo apoptosis, cell cycle arrest (perhaps coupled with DNA repair) is the primary response in differentiated cells. Through these mechanisms, p53 plays an essential role in genetic and tissular homeostasis in response to exogenous or endogenous stresses.

The gene encoding p53, TP53, is inactivated in a majority of human cancers (by mutation, loss of alleles, overexpression of negative regulators such as Mdm2 or expression of viral oncoproteins such as HPV16 E6).[71,72] Loss of p53 function switches off suppressive responses and confer cells a selective advantage for proliferation and escape from apoptosis in the drastic micro-environmental conditions that prevails in many cancers. At the same time, TP53 mutation deregulates a crucial DNA damage checkpoint and makes the cells permissive to the accumulation of further genetic changes during cancer progression.

During inflammatory response, cells produce reactive oxygen and nitrogen species that can damage DNA and induce p53, resulting in extreme case in p53-dependent apoptosis and in tissue damage. These species include hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\bullet}$), and reactive intermediates such as hydroxyl radicals ($OH^{\bullet}$), superoxide ($O_2^{-\bullet}$), and peroxynitrite ($ONOO^-$). Chronic infections and chronic inflammatory diseases, such as ulcerative colitis, result in prolonged oxidative and nitrosive stress and increased cancer risk.[73] A recent study by Staib et al. (2005) has shown that $NO\cdot$ and hypoxia induced similar p53-dependent transcriptional responses in cultured cells, partially different from those elicited by oxidative stress.[74] The biological outcome of this response is essentially quiescent S-phase and G_2-M arrest, rather than apoptosis. Thus, p53 induction by mediators of inflammation may primarily induce a growth suppressive response that contributes to preserve cell viability and genetic integrity, suggesting that p53 may act as an important homeostatic factor during inflammation.

Another link between p53 and inflammation is suggested by studies demonstrating the presence of TP53 mutations in areas of rheumatoid arthritis (RA) synovial tissues. The type of mutation is similar to cancer lesions but data are too limited to fully assess whether the same mutational hotspots are present. This observation is consistent with the notion that induction of p53 by inflammatory stress results in p53-dependent growth suppression, thus providing a selective advantage for clonal expansion to cells that have acquired a mutation in TP53.[75]

COX-2 transcriptional regulation

Many inflammatory factors regulate COX-2 through direct activation of its promoter. The human COX-2 gene, on chromosome 1q25.2-3, encompasses 7.5 kb, contains 10 exons and generates a 4.6 kb mRNA.[1] The 5′ flanking region contains a canonical TATA box and several putative transcription factor binding sites, including among others cAMP-responsive elements (CRE), nuclear factor-kappaB (NF-kappaB), nuclear factor-IL-6 (NFIL-6) activator protein 1 (AP-1), transcription factor 4/ lymphoid enhancer binding protein 1 (TCF4/LEF-1) and c-myb[1,76,77] (Fig. 2). No p53 consensus binding sites (p53 response element) has been identified. Since p53-RE are also found in introns, for example, in MDM2 and DR-5, the sequence of 9 introns of the COX-2 gene has been evaluated and again no p53-RE was identified.[78]

The signaling cascades that regulate COX-2 expression are discussed in several recent reviews.[1,77,79] These cascades include, among others, ERK, JNK/SAPK, p38 MAPK, PKA, cascades initiated by pro-inflammatory cytokines or by activation of TOLL-like receptors. Many of these cascades regulate the binding of NF-kappaB, which is the main regulator of COX-2 expression in response to inflammation. Activation of NF-kappaB often occurs in synergy with NF-IL6 and CRE. Growth signaling cascades primarily activate COX-2 through AP1 and CRE binding sites, as well as through the Wnt/β-catenin signaling pathway, using essentially, but not exclusively, the TCF4/LEF-1 binding site. The role of the c-myb binding sites remains unclear. There are 13 identified binding sites for c-myb in the COX-2 promoter, 8 of which are moderate to high-affinity binding targets.[75] There is concordance between high levels of c-myb and COX-2 in colorectal cancer,

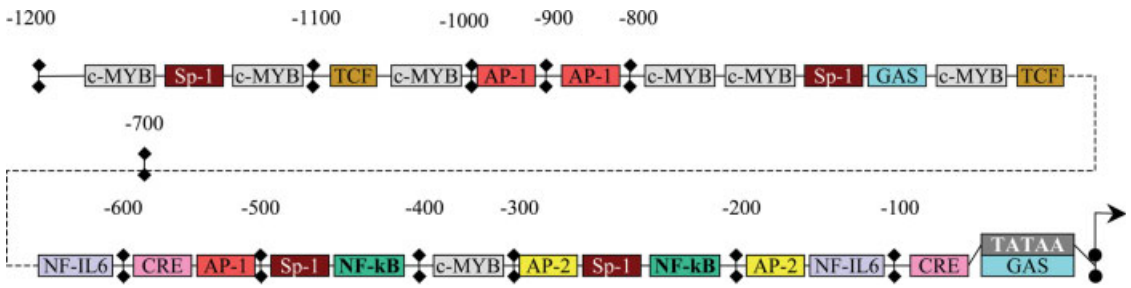

FIGURE 2 – Schematic representation of transcription factor response elements in the human *COX-2* promoter, from −1200 bp to the start site of transcription (not to scale). Several signaling cascades converge onto *COX-2* promoter and may act independently or synergistically to regulate transcription.

suggesting that deregulation of c-myb may play an important role in the expression of COX-2 in tumour tissues.

Additional regulation occurs by modulation of mRNA stability, processing or nuclear export through multiple copies of the translational control element AUUUA located in the first 60 nucleotides of the 3′-Untranslated Region (3′-UTR). These sequences bind a multimeric protein complex in a sequence-specific manner, including members of the protein-RNA complex HuR, TIA-1, TIAR, and the heterogeneous nuclear ribonucleoprotein U (hnRNP U). Activation of these factors may stabilize COX-2 RNA and increase its translation in response to inflammatory or growth promoting signals.[80] Genetic variants (polymorphisms) in the promoter and 3′-UTR of the COX-2 gene modulate risk for prostate, colorectal and non-small-cell lung carcinoma.[81–83]

Down-regulation of COX-2 expression by p53

The first study describing the effect of p53 on COX-2 expression reported a negative regulation.[42] This study compared COX-2 expression and production of prostaglandin E2 in a p53-deficient mouse embryo fibroblasts cell line versus the same cell line engineered to overexpress a murine temperature sensitive (ts) p53 mutant (with a valine instead of alanine at codon 135). In conditions when tsp53 adopts a "wild-type" form (at 32°C), a decrease in levels of COX-2 mRNA and prostaglandin E2 was observed. Deletion constructs showed that, in p53-null cells, promoter activity decreased with the length of the promoter. Nevertheless, repression by tsp53 at 32°C was observed with all deletion constructs, even those containing only the first 40 base pairs 5′ from the transcription start site. Electro-mobility shift assays showed that synthetic wild-p53 coupled to gluthatione S-transferase (GST) competes out TATA-binding protein for binding to a segment of human COX-2 promoter extending from −50 to +52 base pairs. These results suggest that p53 in a wild-type form represses COX-2 by interfering with transcription.

Activation of COX-2 expression by p53

In 2002, Han *et al*.[41] initially identified COX-2 among genes induced by wild-type p53 and DNA damage on gene expression arrays. Using early passages of established human normal mammary cells, cancer cell lines constitutively expressing wild-type p53, or normal mouse embryo fibroblasts, they demonstrated that DNA damaging agents such as mitomycin C or doxorubicin increased COX-2 mRNA and protein in a time- and dose-dependent manner. This effect was not seen in cancer cells null for p53. Furthermore, p53-induced COX-2 expression required activation of the Ras/Raf/MAPK cascade, since dominant-negative Ras or Raf1 mutants suppressed COX-2 induction in response to p53. Induction of COX-2 by p53 appeared to regulate p53-dependent apoptosis in response to DNA damage. First, p53-induced apoptosis was enhanced in COX-2 knock-out cells as compared with wild-type cells. Second, a COX-2 inhibitor, NS-398, enhanced apoptosis induced by DNA damage in normal human cells. Together, these results demonstrate that COX-2 counteracts p53-mediated apoptosis, suggesting that COX-2 plays a role as part of the decision mechanisms by which cells select among p53-dependent responses leading to either survival or apoptosis.

In line with the results of Han *et al*. (2002), we recently reported that wild-type p53 regulated COX-2 expression in esophageal and colon cancer cell lines, and that this effect was dependent upon NF-kappaB.[16] Cells expressing a dominant-negative mutant of I-kappaβ−α were deficient for COX-2 induction by p53, and cooperation between p53 and NF-kappaB was required for activation of COX-2 promoter in response to daunomycin. The mechanism of cooperation between p53 and NF-kappaB involves recruitment of both factors onto a portion of the COX-2 promoter (−700 to +1) encompassing both NF-kappaB binding sites as well as the TATA box binding site. However it is not yet clear whether the 2 proteins assemble in multimeric complexes or whether the cooperation is indirect and involves transcriptional coactivators. Pharmacological inhibition of COX-2 enhanced apoptosis in response to daunomycin, in particular in cells containing active p53, further substantiating that levels and activity of COX-2 can modulate p53-dependent apoptosis.

Based on the results summarized above, there should be a clear correlation between TP53 mutation status and levels of COX-2 expression in tumors. However, a survey of the abundant literature on this question does not allow identifying a simple correlation (Table I). For example, in esophageal cancer, different studies have reported variable results, including correlation with wild-type[16] or mutant TP53 status,[17] or no correlation.[19] With respect to p53 expression as detected by immunohistochemistry, results are extremely discrepant depending on tumor site and histology (Table I).

We reasoned that the correlation between TP53 status and COX-2 expression in tumors might differ depending upon the transcriptional mechanism responsible for COX-2 promoter activation. To test this hypothesis, we analyzed TP53 mutation status in relation with COX-2 expression in cancers of the esophagus.[16] These cancers occurs in 2 major histopathological forms, adenocarcinoma (ADC), that develops from a precursor, inflammatory metaplastic lesion, the Barrett's esophagus, and squamous cell carcinoma (SCC), that develops from the normal mucosa through a classical hyperplasia-dysplasia-carcinoma sequence. As compared with ADC, SCC is less frequently inflammatory, except in subjects from areas in the world where this type of cancer occurs at very high rate, such as for example Northern Iran.[17,19] We found a significant correlation between COX-2 levels and wild-type TP53 status in ADC, but not in SCC. These results suggest that wild-type p53 participates in the up-regulation of COX-2 in ADC and in their precursor lesions, the Barrett's esophagus, but not in SCC.

Regulation of p53 by COX-2

Two studies provide support to the existence of an additional regulatory loop in which COX-2 may interact with p53 and interfere with its function. Corcoran *et al*. (2004)[78] as well as

Choi *et al*. (2005)[84] have shown that, after up-regulation by p53, COX-2 can in turn inhibit p53-dependent transcriptional activity. Overexpression of COX-2 down-regulates the expression of several p53 target genes and decreases p53-dependent apoptosis.[84] In contrast, selective inhibition of COX-2 potentiates p53-induced apoptosis.[78] This effect may be due to a physical interaction between p53 and COX-2, in which COX-2 interferes with p53 binding to target promoters. The direct binding between p53 and COX-2 has been shown *in vivo* (co-immunoprecipitation assay) and *in vitro* (GST-pull down assay), with potential physiological relevance.[78,84] However, it cannot be excluded that the effect of COX-2 on p53-dependent transcription is indirect, involving increased production of prostaglandins and down-regulation of p53 function through more complex signaling cascades. Recently, Faour *et al*. (2006) have reported that PGE_2 induced a time-dependent increase in p53 Serine 15 phosphorylation, through activation of p38MAP kinase, stimulating p53 transactivational activity in synovial fibroblasts.[85] This observation suggests that COX-2-derived prostaglandins may be implicated in regulating p53 function.

Physiological and pathological scenarios for cross-talks between p53 and COX-2

The data summarized above demonstrate that p53 and COX-2 entertain cross-talks in which each factor controls the activity of the other. Depending upon conditions of p53 and COX-2 expression, p53 may either induce or decrease COX-2 expression, and COX-2 may in turn down-regulate p53 transcription. Furthermore, we have shown that induction of COX-2 expression requires NF-kappaB, which has also been shown to bind and down-regulate human TP53 promoter. In the following paragraphs, we propose different scenarios in which these cross-talks may play a physiological or a pathological role (Fig. 3).

In inflammatory tissues

In inflammatory lesions, multiple signaling pathways are activated simultaneously, including pathways leading to activation of both NF-kappaB (mainly in response to inflammatory cytokines) and p53 (mainly as a consequence of DNA damage by reactive oxygen and nitrogen species). Cells exposed to these stresses are faced with delicate life and death decisions and need to develop mechanisms to keep in check the capacity of p53 to induce apoptosis, which would otherwise result into acute cell and tissue destruction. In this context, cooperation between p53 and NF-kappaB to up-regulate COX-2 provides a failsafe mechanism by which cells protect themselves against p53-induced apoptosis. This control involves first p53 cooperating with NF-kappaB to transactivate COX-2 in response to DNA damage, and second COX-2 downregulating p53 transcriptional activity through direct or indirect mechanisms (Fig. 3.1). Since NF-kappaB can activate COX-2 expression in the absence of p53, the specific role of p53 may be to provide a link between COX-2 activation and DNA damage. In the event of extreme p53 accumulation and activation, wild-type p53 may down-regulate COX-2 expression by competing out TATA binding proteins, providing a mechanism that switches off COX-2 function and restore p53-dependent apoptosis in cells undergoing irreparable DNA damage. Thus, this cross-talk may contribute to a physiopathological mechanism of homeostasis in cells exposed to inflammation.

In precursor and cancer lesions

Precursor and early cancer lesions are characterized by enhanced proliferative capacity and escape from apoptosis. At least 2 distinct mechanisms of upregulation of COX-2 expression may operate in such lesions (Fig. 3.2). In inflammatory precursor lesions, such as Barrett's esophagus, the same regulatory loop as described above may keep alive cells exposed to chronic, inflammatory stress (scenario 2.1). Such cells would thus retain a functional regulatory cross-talk between wild-type p53 and COX-2 as a survival mechanism that facilitates progression towards neoplasia. Tumors occurring in this context may, in their early stages, often retain wild-type TP53 in conjunction with high COX-2 expression. This situation prevails in lesions such as ADC of the esophagus or adenoma of the colon. In later stages of tumor progression, wild-type p53 may not be required to maintain a COX-2-dependent protection against apoptosis, either because COX-2 becomes activated by mechanisms independent of p53 and NF-kappaB, or because cells have acquired other modifications allowing them to escape apoptosis.

The alternative scenario may occur in lesions in which inflammatory stress is an epiphenomenon rather than a driving mechanism in cancer development (scenario 2.2). In such lesions, increased expression of COX-2 may occur primarily as a consequence of promoter activation by growth promoting signaling cascades such as those involving Wnt/βCatenin, K-ras or c-Myb. Thus, COX-2 activation would be largely independent of NF-kappaB and p53, and COX-2 expression may coexist with TP53 mutation in the same cell. Both events may have synergistic effects in inhibiting apoptosis, so that tumors in which both deregulations occur may have a particularly dismal prognosis. This situation may prevail in many squamous cancers, as well as in lesions in which TP53 mutation occurs prior to the development of a lesion as a result of intense exposure to environmental carcinogens. This may be the case, for example, in lung cancers of smokers, irrespective of the histological type. However, it must be considered that activation of Cox-2 promoter by growth signaling cascades may be relevant in most tumors, including those where inflammatory stress is at hand.

Conclusions

Implications for therapeutic and preventive strategies based on COX-2 inhibition

Despite apparently contradictory effects, most of published data on cross-talks between p53 and COX-2 can be rationalized into 2 compatible scenarios in which deregulation of COX-2 may occur either as a consequence of the cooperation between p53 and NF-kappaB in cells exposed to damaging reactive species produced during inflammation, or of the excessive stimulation of growth signaling cascades resulting from oncogenic stress. Although not necessarily mutually exclusive, these 2 scenarios may account for the diverse patterns of COX-2 expression in relation with TP53 gene status in tumor cells. They have also important implications for the use of pharmacological inhibitors of COX-2 in tumor prevention and therapy.

The development of specific COX-2 inhibitors has raised expectations for the treatment of chronic inflammation and for the development of chemopreventive cancer interventions (reviewed in Ref. 86). These inhibitors belong to 3 major chemical classes, including (*i*) 1,2-diarylcyclopentenes (so-called tricyclic compounds), such as celecoxib and rofecoxib; (*ii*) methanesulphonamide compounds, such as NS-398 and nimesulide; and (*iii*) NSAIDS-derivatives, such as meloxicam and etodolac. These inhibitors largely avoid side effects associated with the use of conventional NSAIDs that also inhibit COX-1, such as gastrointestinal bleeding, reduction of renal blood flow, and platelet dysfunction. In 2000, a randomized prospective study reported that celecoxib significantly reduced the number of intestinal polyps in patients with FAP, leading to the approval of this drug for clinical use in cancer prevention in FAP patients.[87] A broad protective effect was demonstrated further on upper gastrointestinal tumors. However, these trials also identified an increased risk of cardiovascular adverse events in patients treated with rofecoxib, leading to its withdrawal from the market in 2004 and 2005. Despite this major concern, selective COX-2 inhibitors have demonstrated significant anti-cancer effects, providing important lessons for the development of further approaches to pharmacologically modulate COX-2 expression and activity.

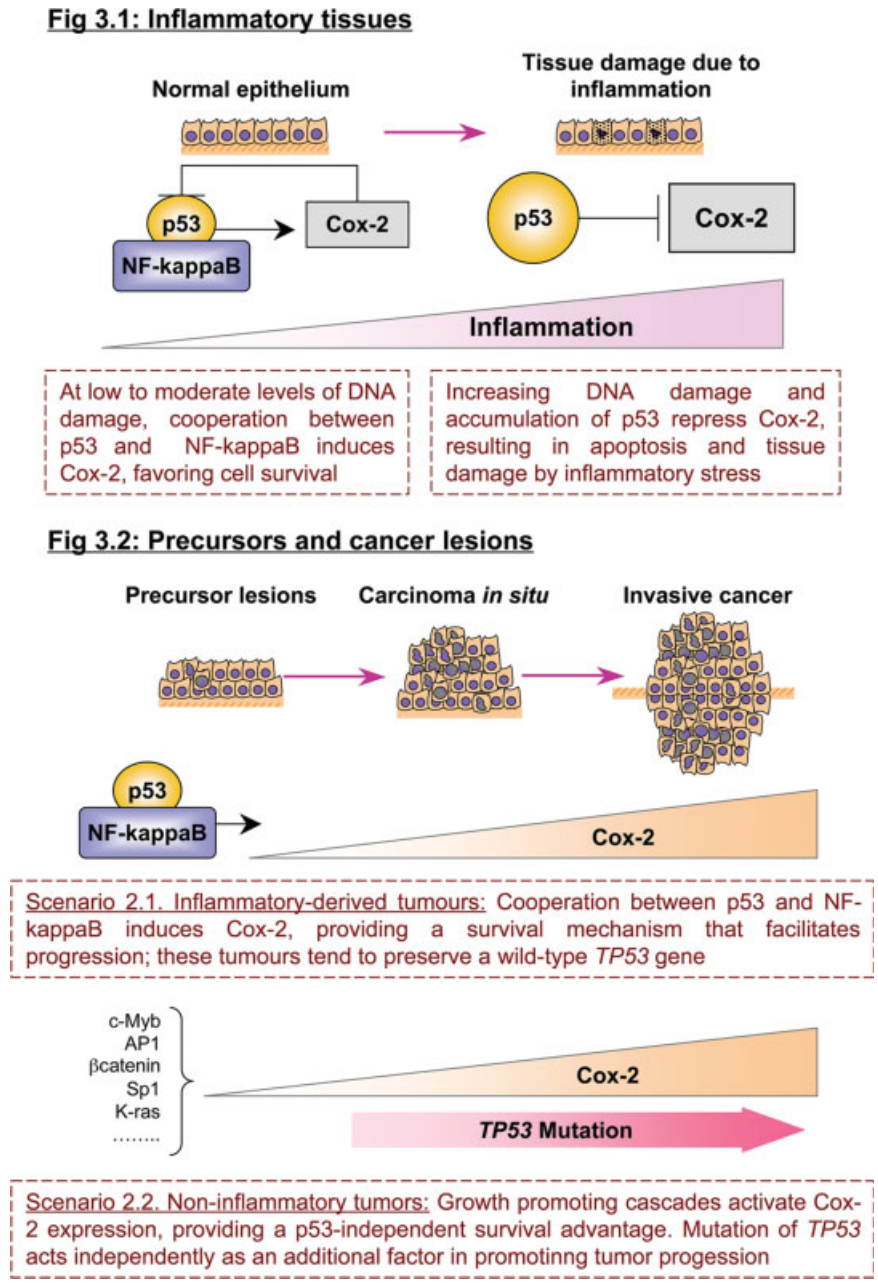

FIGURE 3 – Two different scenarios explaining the variable patterns of p53 and COX-2 expression in inflammatory tissues, precursor and cancer lesions.

The 2 scenarios outlined above have different implications for the use of COX-2 inhibitors in cancer management. In the "inflammatory" scenario, COX-2 inhibitors may mediate chemopreventive effects by switching off COX-2 activity and restoring p53-dependent apoptosis in precursor lesions, leading to their elimination. This effect may explain the impact of coxibs in patients with FAP. In the "constitutive proliferation" scenario, however, switching off COX-2 may have relatively little benefits in tumors that have lost the capacity to develop a p53-dependent apoptotic response. On the other hand, in tumors with functional, wild-type p53, the effect of COX-2 inhibitors may be enhanced by simultaneous activation of p53 using conventional, chemotherapeutic drugs. Lau *et al*. (2006) have shown that COX-2 inhibitors are able to enhance chemosensitivity in neuroblastoma *via* downregulation of Mdm2 and augmentation of p53 stability, nuclear localization and activity.[88] Thus, in this context, COX-2 inhibitors may be more useful as modulators of chemotherapy in cancer patients than as chemopreventive drugs in subjects with precursor lesions. Recently, a study conducted in non-small-cell lung cancer patients has shown that treatment with taxane-based chemotherapy led to increased amount of COX-2 and PGE_2. Cotreatment with celecoxib abrogated the increase in levels of PGE_2 induced by chemotherapy.[89] It has also been shown that COX-2 may interact considerably with the EGFR signal pathways.[90,91] Clinical trials are ongoing to assess whether combining low doses of COX-2 and EGFR tyrosine kinase inhibitors are more effective than either agent alone.[92]

In conclusion, cross-talks between p53 and COX-2 illustrate how 2 effectors acting in distinct signaling cascades and exerting opposite functional effect, have developed several mechanisms of

cooperation to regulate the delicate balance between cell life and death. These mechanisms provide a paradigm for how cells handle these crucial decisions in inflammation, in response to genotoxic stress, and during normal physiological processes such as differentiation or senescence.

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