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Non-steroidal anti-inflammatory drugs to potentiate chemotherapy effects: From lab to clinic

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Contents

Abstract

Most solid tumors express the cyclooxygenase-2 (COX-2) protein, a target of NSAIDs. COX-2 overexpression in tumorsis considered a predictor of more advanced stage disease and of worse prognosis in a number of studies investigating solid malignancies. Therefore, NSAIDs are evaluated as anti-cancer drugs. NSAIDs inhibit proliferation, invasiveness of tumors, and angiogenesis and overcome apoptosis resistance in a COX-2 dependent and independent manner. This review will focus on the rationale behind NSAIDs, including selective COX-2 inhibitors, in combination with conventional chemotherapeutic drugs or novel molecular targeted drugs. Studies investigating anti-cancer effects of

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NSAIDs on cell lines and xenograft models have shown modulation of the Akt, NF-κB, tyrosine kinase and the death receptor-mediated apoptosis pathways. COX-2 expression in tumors is not yet used as biomarker in the clinic. Despite the increased risk on cardiovascular toxicity induced by selective COX-2 inhibitors, several ongoing clinical trials are still investigating the therapeutic benefits of NSAIDs in oncology. The anti-tumor effects in these trials balanced with the side effects data will define the precise role of selective COX-2 inhibitors in the treatment of cancer patients.

Keywords: NSAIDs; Cyclooxygenase; Chemotherapy

1. Introduction

Solid tumors are one of the leading causes of death in the Western countries with an increasing number of cancer patients every year. Although the prognosis of these patients has improved the last decade, there is still a need for novel treatment modalities. Therefore, new targets for anticancer treatments are sought. From large retrospective and prospective population-based studies it was learned that regular use of both non-selective non-steroidal anti-inflammatory drugs (NSAIDs), and selective cyclooxygenase-2 (COX-2) inhibitors is associated with an important decreased incidence of colorectal, breast, bladder, prostate as well as lung cancer [1–7].

Preclinical data suggested that the inhibition of COX-2 is responsible for this decrease in cancer incidence. In addition there is increasing evidence that selective and non-selective COX-2 inhibitors have COX-2 independent effects that can account for the anti-tumor effect of these agents. Moreover, data from cell lines and animal models have shown that NSAIDs in combination with chemotherapy enhances efficacy or can even circumvent drug resistance. Similar findings have been described for NSAIDs in combination with novel molecular targeted therapeutics. This review will focus on potential benefits of selective or non-selective COX-2 inhibitors added to conventional or experimental cancer treatments. COX-2 dependent and COX independent mechanisms for this sensitization will be described.

2. Physiological function of cyclooxygenases

2.1. Cyclooxygenases

Cyclooxygenase (COX) is the enzyme that catalyses the conversion from arachidonic acid to prostaglandins (PGs) [8]. There are three isoforms of COX, COX-1 and COX-3. The *COX-1* gene was cloned by three separate groups in 1988 [9–11]. In 1991, Xie et al. discovered an inducible *COX* gene named *COX-2* [12], while *COX-3* was discovered in 2002, being a splice variant of *COX-1* [13]. COX-1 is involved in maintenance of the gastric mucosa, in regulation of renal blood flow in the afferent vessels of the kidney and in regulation of platelet aggregation. The second isoform, COX-2 is an inducible isoform, which is involved in inflammation and tumorigenesis. The third isoform, COX-3 is involved in anti-inflammatory reactions and the production of anti-inflammatory PGs [13].

COX-2 has two functionalities, i.e. cyclooxygenase activity to oxydate arachidonic acid and peroxidase activity to convert chemicals, from for instance tobacco smoke, into highly reactive mutagens that can bind to DNA [14]. PGs and thromboxane A_2 are the end products of the conversion of arachidonic acid by COX. The process of converting arachidonic acid into PGs and thromboxane is initiated by the conversion of arachidonic acid into prostaglandin H_2 (PGH_2). PGH_2 is converted by tissue specific isomerases into five primary active structurally related PGs. These PGs include PGE_2, PGD, $PGF_{2\alpha}$, PGI_2 and thromboxane A_2 (TXA_2) via tissue specific PG synthetases [15–18]. Eight types of prostaglandin receptors have been recognized. All are membrane-bound G-protein coupled receptors encoded by different genes. COX-2 expression is induced by several mitogenic and proinflammatory stimuli including basic fibroblast growth factor [19], transforming growth factor β1 [20], epidermal growth factor [21], vascular endothelial growth factor (VEGF) and tumor necrosis factor alpha (TNFα), lipopolysaccharide, and interleukins 1α and 1β [22]. Transcriptional upregulation of COX-2 can occur by NF-κB, cAMP response element, nuclear factor-interleukin 6, PEA3, nuclear factor of activated T cells 1, Ras/Raf/MAPK, AP-2, and SP-1 [23–27].

2.2. COX knock-out mice models

Knock-out mice have been made to investigate the physiologic function of COX-1 and COX-2. Mice lacking COX-2 developed cardiac fibrosis, nephropathy and peritonitis. The causes of death of mice 3 weeks after birth were peritonitis or kidney malfunction [28]. Male COX-2 null mice were fertile while females were infertile. In all COX-2 null mice kidney morphology was abnormal. The kidneys were paler and smaller than those of COX-2 wild-type mice with less and poorly developed glomeruli. In addition, renal tubuli were more dilated and atrophied compared to those in wild-type mice [28].

2.3. NSAIDs

Most research with respect to COX-1 and COX-2 protein function, however, was performed with NSAIDs. There is

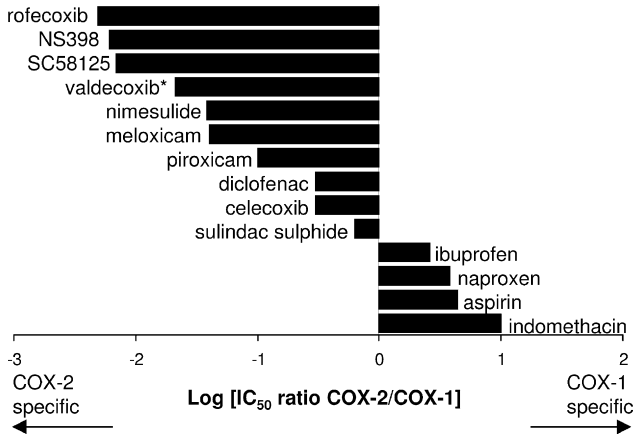

Fig. 1. COX-1 and COX-2 selectivity of different drugs as measured by the William Harvey Human Modified Whole Blood Assay [33]. *Measured by using stimulated peripheral human monocytes [34].

60% homology between the amino acid structures of COX-1 and COX-2 [9–11]. The COX-2 cyclooxygenase active site has a slightly larger hydrophobic side-pocket than the active site of COX-1, which can be used to make COX-2 selective inhibitors [29–31]. Selective COX-2 inhibitors were designed to prevent peptic ulcer, gastrointestinal bleeding, and/or perforation of gastroduodenal ulcers which is associated with prolonged use of NSAIDs. The NSAID aspirin inhibits cyclooxygenases by covalent binding to the active site of the enzyme, while all other NSAIDs and COX-2 inhibitors inhibit cyclooxygenase enzymatic activity by competing with the substrate for the active site. Although aspirin binds to COX-1 and COX-2 at a similar site, arachidonic acid can still be converted by COX-2 due to the larger active site of COX-2 [32]. The relative potencies as inhibitors of COX-1 and COX-2 for non-selective NSAIDs as well as selective COX-2 inhibitors are illustrated in Fig. 1 [33–35]. Selectivity for either COX-1 or COX-2 can be observed at relatively low concentrations of the COX inhibitor. All COX inhibitors, however, can inhibit both COX-1 and COX-2 at higher concentrations because of the homology of the active sites of these enzymes. The anti-cancer effects of NSAIDs are often observed at concentrations that exceed the COX-2 inhibitory concentration. Therefore, these anti-cancer effects are considered to be partially COX-2 independent. For instance, COX-2 independent apoptosis induction of celecoxib has been described in HT29 colon cancer cells. Celecoxib-mediated apoptosis is induced in these cells by 3-phosphoinositide-dependent kinase (PDK1) inhibition. PDK1 can phosphorylate Akt and therefore celecoxib is an indirect inhibitor of the Akt pathway. To prove that PDK1 is the most important target of celecoxib-mediated apoptosis a constitutive active PDK1 mutant was introduced in the HT29 cells. Overexpression of the constitutive active mutant of PDK1 ($PDK1^{A280V}$) inhibited apoptosis as potent as the pancaspase inhibitor zVAD [36].

3. Potential role of COX-2 in cancer development

3.1. COX-2 and carcinogenesis

The role of COX-2 and therefore NSAIDs in cancer development and cancer chemoprevention has been extensively reviewed in the past [37–40]. To investigate the role of COX-2 in cancer development in more detail a number of COX-2 knock-out models were used. In heterozygous adenomatous polyposis coli (*Apc*) knock-out mice all the animals develop intestinal polyps [41]. The role of COX-2 expression in this polyp formation was investigated by studying double knock-out mice with a heterozygous *Apc* knock-out and a homozygous COX-2 knock-out genotype. These double knock-out mice developed 86% less polyps compared to the COX-2 wild-type mice [42]. This effect was dose dependent because the COX-2 heterozygous knock-out mice showed an intermediate reduction of polyp formation. Another mouse model addressing the role of COX expression in carcinogenesis is the multiple intestinal neoplasia (*Min*) mouse model. The *Min* mice also have a chemically induced non-sense mutation in the *Apc* gene and all these mice develop intestinal neoplasia, although the polyp formation is less than in *Apc* knock-out mice. When *Min* mice are crossed with COX-1 or COX-2 null mice on a C57Bl/6 background, polyp formation was decreased by 70–80% [43,44]. Therefore, it can be concluded that COX-1, as well as COX-2, play a key role in intestinal carcinogenesis.

Overexpression of COX-2 in transgenic mice using a mammary gland specific murine mammary tumor virus promoter caused mice to develop mammary cancer after successive rounds of pregnancy. This did not happen in virgin females, probably because proliferating epithelial cells in the pregnant mammary gland are more susceptible to mutagenic events in critical tumor suppressor genes. Mice that received the empty vector containing the MMV promoter did not develop mammary tumors [45].

3.2. COX-2 and tumor angiogenesis

Neoangiogenesis is a crucial event in tumorigenesis and development of metastases. Tumor growth beyond 2–3 mm in size requires neovascularization and inhibition of angiogenesis at this stage is therefore a potential important target in cancer therapy. One of the first observations indicating that COX proteins may be of importance in angiogenesis was described in a study published in 1997 [46]. Both COX-1 and COX-2 are involved in tumor vascularization. COX inhibitors can directly affect angiogenesis [47]. PGI_2 regulates endothelial sprouting as well as VEGF-induced vascular permeability [48–50]. PGE_2 can induce VEGF production by activation of two different pathways, the ERK2/JNK1 pathway and by translocation of hypoxia-inducible factor from the cytosol to the nucleus [51,52].

Angiogenesis was blocked in nude mice carrying Colon-26 cells after treatment with diclofenac. There is mounting

evidence that PGs participate in angiogenesis, regulating the production of proangiogenic factors such as VEGF [48,53]. COX-1 mediated induction of endothelial cell tube formation as was shown by Tsujii et al. [54]. The increased PG production can stimulate non-malignant stromal cells to produce VEGF. The VEGF than stimulates local endothelial cells to proliferate into the tumor [51]. Interestingly, stromal cells from COX-2 knock-out mice show a 94% reduction in VEGF levels compared to wild-type fibroblasts. Wild-type fibroblasts exposed to a selective COX-2 inhibitor also had a 92% lower VEGF expression [55].

High PGE_2 levels have been associated with metastatic disease in breast cancer patients due to increased proliferation and angiogenesis, which also suggests autocrine and paracrine signaling [56,57]. A study in breast cancers in which COX-2 expression was compared to VEGF expression by confocal immuofluorescence analysis showed a positive correlation between COX-2 and VEGF expression [58].

3.3. COX-2 and tumor progression

Progression of tumors can be induced by hormones such as estrogens and androgens, but also by PGs or by down-regulation of anti-apoptotic proteins. In breast cancer PGE_2 overexpression in the tumor induces aromatase production in human adipose stromal cells in the breast and concomitantly estrogen production, which stimulates tumor proliferation [59]. In human breast cancers a strong correlation between COX-2 expression and cytochrome P450 enzyme aromatase (CYP 19) was found [60]. Thus, COX-2 may be the cause of progression of estrogen-dependent breast cancer either directly by stimulating tumor cell proliferation, or indirectly by upregulating aromatase activity [60]. The LS-174 colorectal carcinoma cell line, which does not generate detectable prostaglandins, increases DNA synthesis and growth after addition of PGE_2 to the culture medium [61]. These effects were most likely caused by activation of the PI3K/Akt pathway as PI3K/Akt inhibitors also inhibited the PGE_2-mediated increased tumor growth. This activation of the PI3K/Akt in turn is most likely regulated by PG-coupled G-proteins. In colon cancer cell lines COX-2 also provides cells with a more invasive and metastatic phenotype compared to cells with less COX-2 expression as was observed in COX-2 transfected Caco-2 cells [62]. With the same technique, invasion caused by COX-2 overexpression is investigated in the MDA-231 breast cancer cell line. COX-2 overexpression in the MDA-231 breast cancer cell line enhanced cell motility and invasiveness as was investigated with Matrigel invasion experiments thus suggesting a mechanism of COX-2 stimulated metastasis [63].

4. NSAIDs and radiotherapy

Upregulation of prostaglandin synthesis after irradiation is a tumor protective effect. Selective COX-2 inhibitors have also been described to enhance radiotherapy efficacy primarily by inhibition of angiogenesis [55]. This is a COX-2 dependent effect because neutralization of COX-2 derived PGE_2 has the same effect as celecoxib exposure *in vivo* in Col26 colon cancer cells. In this study, tumor vasculature was measured with contrast magnetic resonance imaging (MRI) [64]. Apart from its involvement in angiogenesis, COX-2 overexpression can also directly affect radiosensitivity of tumor cells as shown in eight oral squamous cell carcinoma cell lines [65]. The level of the COX-2 expression in these oral squamous cell carcinoma cell lines correlated with increased tumor radiation resistance [65]. The COX-2 expressing NCI-H460 and A549 cells were sensitized for radiotherapy by exposure to celecoxib. Downregulation of COX-2 reduced the radiation-enhancing effects of celecoxib in A549 cells. In contrast, the COX-2 non-expressing MCF-7 and HCT-116 cells were not radiosensitized by celecoxib. HCT-116 cells, transfected with COX-2 expression vector, were also radiosensitized by celecoxib, demonstrating that radiosensitization by celecoxib occurs in a COX-2 dependent manner. Reduced production of PGE_2 after celecoxib treatment was not instrumental, since the addition of PGE_2 had no effect on the radiosensitizing effects of celecoxib. Thus, the question remains via which mechanism COX-2 inhibition by celecoxib affect radiosensitivity [66].

5. NSAIDs and chemotherapeutic agents

5.1. Combination of NSAIDs and chemotherapy in cell line models

5.1.1. NSAIDs to bypass conventional chemotherapy resistance

One of the first steps to investigate the efficacy of NSAIDs in cancer therapy is to combine them with conventional chemotherapeutic agents. Part of the rationale for combining NSAIDs with chemotherapy involves circumvention of chemotherapy resistance mechanisms.

The Bcl-2 family of pro and anti-apoptotic proteins promotes or inhibits apoptosis at the mitochondrial level. Bcl-2 family members confer a clinically important resistance to chemotherapeutic agents in a number of hematologic and solid malignancies, including acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, chronic lymphoblastic leukemia, multiple myeloma, prostate cancer, malignant brain tumors, and neuroblastoma [67–72]. Down-regulation of Bcl-2 increases toxicity for chemotherapeutic agents in a number of tumor cell lines [73,74]. NSAIDs such as the selective COX-2 inhibitors SC-58125 and NS-398 can down-regulate Bcl-2 and subsequently induce apoptosis in colon and prostate cancer cell lines [75]. On the other hand, an increase in pro-apoptotic Bax protein and a decrease in Bcl-X_L protein can also induce apoptosis as shown in HCT116 colorectal carcinoma cells [76]. Celecoxib, aspirin and indomethacin could induce apoptosis by Bak and Bax

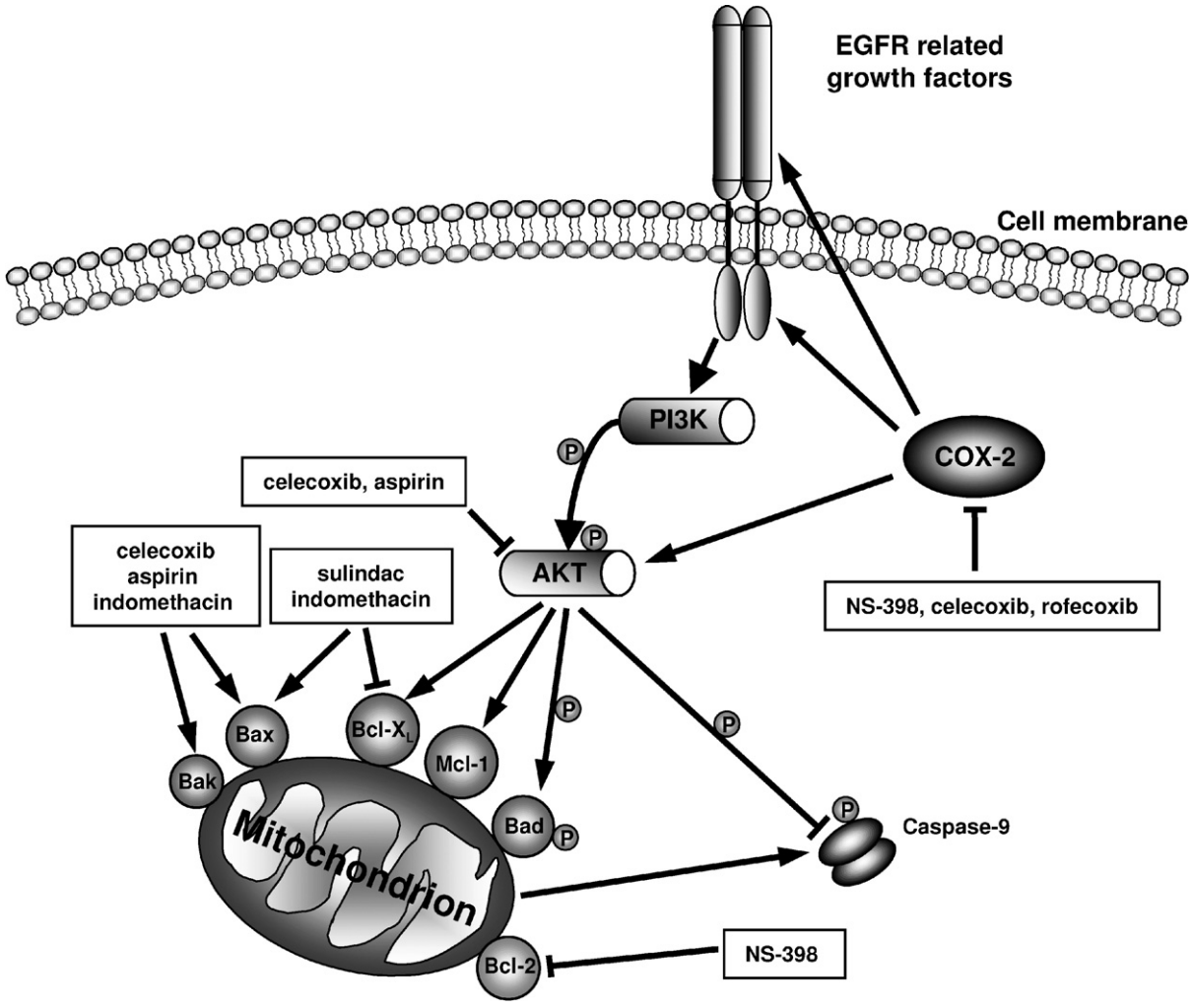

Fig. 2. Akt can be upregulated by COX-2, while both Akt and COX-2 are inhibited by NSAIDs. Inhibition of Akt prevents phosphorylation and inactivation of Bad and caspase-9 resulting in decreased apoptosis. Different NSAIDs can modulate expression of Bcl-2 family members. Modulation of Bcl-2 family members involves downregulation of anti-apoptotic members and upregulation of pro-apoptotic members.

upregulation, mitochondrial membrane potential loss and activation of caspase-3 [77–79] (Fig. 2).

There is also an indirect way of targeting the Bcl-2 family of anti-apoptosis proteins namely by inhibiting Akt signaling. Akt/PKB is a serine/threonine protein kinase that functions as a critical regulator of cell survival and proliferation. In recent years, it has been demonstrated that PI3K/Akt signaling components are frequently altered in human cancers. Increased extracellular signaling by growth factors and increased signaling of intracellular components of the Akt pathway such as Ras are frequently observed. The third mechanism of increased Akt signaling is the decreased activity of Akt regulatory proteins. Akt phosphorylation is associated with phosphorylation and thus inactivation of the pro-apoptotic proteins Bad and caspase-9. Phosphorylated Bad cannot bind to Bcl-2, while phosphorylated caspase-9 cannot be activated resulting in a reduced activity of mitochondrial apoptosis [80,81]. Inhibition of Akt phosphorylation by celecoxib and aspirin leads to apoptosis in the human A549 non-small cell lung cancer cell line [82]. In an *in vivo* model of spontaneous metastatic breast cancer celecoxib-induced apoptosis correlated with a significant decrease in Akt activation [83]. Celecoxib also suppressed Akt phosphorylation and kinase activity in cultured human C611B cholangiocarcinoma cells, which correlated with Bax translocation to mitochondria, cytochrome *c* release into the cytosol, followed by activation of caspase-9 and caspase-3. Addition of PGE_2 to these cells blocked the apoptotic actions of celecoxib [84]. In another study with cholangiocarcinoma cell lines celecoxib-induced reduction of Akt phosphorylation, whereas the absolute cellular Bcl-2 and Bax levels remained unaltered. Akt protein was inhibited by LY294002 decreasing the viability of these cell lines. These effect were partially COX-2 mediated because after addition of PGE_2 to the growth medium apoptosis induction was decreased [85]. Stable transfection of the human lung adenocarcinoma cell line CL1.0 with the

COX-2 gene results in activation of the PI3K/Akt-dependent pathway, which promotes cell survival by PI3K/Akt-dependent up-regulation of the Mcl-1 protein level (Fig. 2) [86].

The inhibitors of apoptosis (IAPs) are a family of proteins that function as intrinsic regulators of the caspase cascade. Members of this family include, survivin, c-IAP1, c-IAP2, XIAP, livin, NAIP, ILP-2 and Bruce. These proteins have been identified to regulate the activity of both initiator (caspase-9) and effector caspases (caspase-3 and -7). IAPs have also been implicated in decreased tumor responses in cytotoxic therapy by several experimental studies. In ovarian cancer cell lines it has been shown that the ability of cisplatin to down-regulate XIAP may be an important determinant of chemosensitivity. Antisense downregulation of XIAP expression increased cisplatin sensitivity in cisplatin sensitive cell lines and to a lesser extend in cisplatin resistant cell lines [87–89]. XIAP overexpression in the human myeloid leukemia cell line U-937 suppressed apoptosis *in vitro* following treatment with 1-[β-D-arabinofuranosyl]cytosine [90]. In this type of chemotherapy resistance NSAIDs can enhance therapy efficacy by modulating IAP expression [91]. In intestinal epithelial cells PG signaling protects normal and transformed intestinal epithelial cells from apoptosis by, rapid induction of cellular inhibitor of apoptosis protein (c-IAP) 2 and delayed induction of another member of the IAP family, livin. Inhibition of COX-2 in cells overexpressing this enzyme decreases c-IAP2 expression and promotes apoptosis, both of which are reversible by PGE2 addition [91]. COX-2 protein overexpression in non-small cell lung cancer (NSCLC) cell lines stabilizes survivin by decreasing ubiquitination of survivin, thus increasing resistance to apoptosis [92]. This effect was also described after exogenous PGE_2 exposure of COX-2 non-overexpressing tumor cells [92,93]. Exposure of HT-29 colon carcinoma cells to sulindac decreased survivin mRNA and protein expression [94].

NF-κB is a member of the Rel family of transcription factors usually present in the cytoplasm as hetero or homodimers. NF-κB exerts its anti-apoptotic or apoptotic effects through transcriptional activation or inactivation of specific genes, a process that only occurs in the nucleus. NF-κB activity is tightly controlled through regulation of NF-κB translocation to the nucleus. In the cytoplasm, NF-κB is bound to inhibitor molecules alpha (IκBα) or I kappa B beta (IκBβ). When bound to IκB, NF-κB cannot enter into the nucleus. IκB can be phosphorylated by IκB kinase, which results in proteasomal degradation of IκB and subsequent translocation of NF-κB to the nucleus. Constitutive NF-κB activation, observed in many malignant tumors, protects the cells from apoptotic stimuli, such as anti-cancer treatments. NF-κB can also confer resistance to cytotoxic therapy for instance by upregulation of anti-apoptotic proteins such as c-IAP1, c-IAP2, XIAP, TRAF1, TRAF2, Bfl-1/A1, Bcl-X_L, or c-FLIP (Fig. 3) [95]. Interestingly numerous factors including ionizing radiation and certain chemotherapy agents can induce NF-κB activation. Therefore, downregulation of NF-κB activity could be a target for chemosensitization. *In vivo* administration of NF-κB siRNA by adenoviral delivery reduces HCT116 tumor formation in xenograft models in the presence but not the absence of the chemotherapeutic drug irinotecan [96]. Xenografts of HT1080 fibrosarcoma cells transfected with a modified IκBα, which is a NF-κB inhibitory protein, are more sensitive to irinotecan and TNF exposure [97]. Thus, NF-κB inhibition can enhance *in vivo* sensitivity to chemotherapeutic agents. Inhibition of NF-κB is one of the COX-2 independent mechanisms by which NSAIDs induce apoptosis [98]. Aspirin inhibits IκB kinase (IKKβ). This prevents the phosphorylation and subsequent degradation of IκB. Due to an increase in IκB/NF-κB complexes, NF-κB is trapped in the cytoplasm and transcriptionally inactive [99] (Fig. 3). A similar effect, i.e. inhibition of IKKβ activity, was reported for sulindac [100]. Unlike aspirin and sulindac, the COX-2 inhibitor SC236 affected neither the phosphorylation, degradation, nor expression of IκB-α. Instead, SC236 worked directly on NF-κB suppressing the nuclear translocation of NF-κB [101]. Celecoxib suppresses constitutively active NF-κB and drug-induced activation of NF-κB by a number of agents, such as TNF, phorbol ester, okadaic acid, lipopolysaccharide (LPS), and IL-1β without cell type specificity [102].

NSAIDs can also decrease chemotherapy resistance by inhibition of members of the ATP binding cassette family of drug transporters. In COR L23R, DLKP and A549 human lung cancer cell lines anthracycline resistance was circumvented by co-exposure to NSAIDs such as meclofenamic acid, diclofenac, naproxen, fenoprofen, phenylbutazone, flufenamic acid, flurbiprofen, ibuprofen and ketoprofen [103]. Celecoxib can decrease, in a COX-2 independent manner, multidrug resistance protein-1 (MRP1) expression in A549 lung cancer cells and therefore increase anthracycline sensitivity [104]. In addition, in HL-60 human leukemia cells incubation with the NSAID meloxicam also resulted in decreased MRP1 expression. This downregulation, however, is COX-2 dependent, because preincubation of these cells with PGE2 neutralized the increase in chemotherapy sensitivity [105]. In NCI-H460 lung cancer bearing mice, sulindac increased the anti-cancer effect of doxorubicin [106].

The modulation of NSAIDs on chemotherapy resistance mentioned in the former section was mostly anthracycline resistance. Modulation of platinum containing chemotherapeutic agents occurs through different pathways. For instance the selective COX-2 inhibitor JTE-522 increases cisplatin sensitivity by decreasing Bcl-2 expression in T24 bladder cancer cells [107]. Oxaliplatin and the NSAID etodolac decreased survivin expression and increased death and growth inhibition in RKO colon cancer cells [108]. On the other hand NSAIDs can also inhibit platinum containing chemotherapy efficacy. Nimesulide inhibits the cytotoxic effect of cisplatin in head and neck squamous cell cancer cell lines [109].

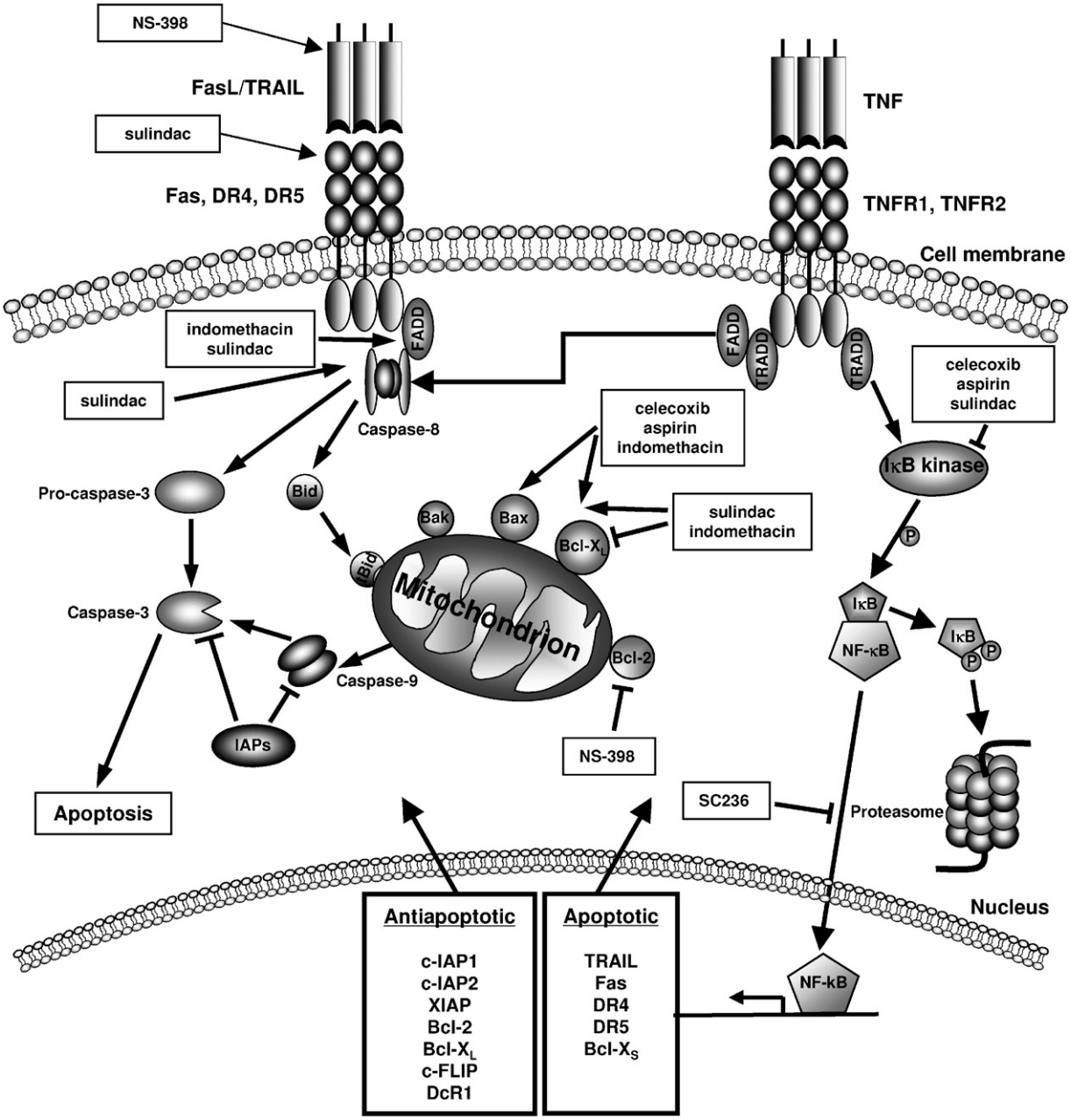

Fig. 3. Death receptor-mediated apoptosis is facilitated at different levels by NSAIDs. NS-398 increases FasL expression, sulindac sulfide increases DR5 expression and indomethacin and sulindac sulfide can activate the intracellular part of the death-inducing signaling complex. Inhibition of IκB kinases by selective and non-selective COX inhibitors results in decreased NF-κB in the nucleus and subsequently in a decreased transcription of pro- and anti-apoptosis proteins.

5.1.2. NSAIDs combined with novel molecular targeted therapeutics

Recent data on combinations of NSAIDs and novel molecular targeted anti-cancer therapeutics have provided evidence that there are cross-talks between pathways targeted by COX inhibitors and other molecular targeted therapies. Activation of Her-2/Her-3 tyrosine kinase signaling in colorectal cancer cell lines resulted in elevated COX-2 expression levels and subsequently in an increase in PGE_2 production [110,111]. Upregulation of COX-2 protein mRNA and expression has also been observed in a breast cancer cell. A complex including nuclear Her-2 binds at a specific nucleotide sequence of the COX-2 promoter region and hereby promotes transcription [112]. Exposure of HCA-7 rectal carcinoma cells to the combination of celecoxib and trastuzumab decreased growth *in vitro* as well as in a xenograft model. Therefore, targeting COX-2 and HER-2 with a combination of NSAIDs and anti-HER-2 antibody such as trastuzumab or

a HER-2 tyrosine kinase inhibitor is a rational combination [113].

The epidermal growth factor receptor (EGFR; erbB1) is a member of the tyrosine kinase receptor family, which includes HER-2/neu (erbB2), erbB3, and erbB4 [114,115]. The ErbB receptors are present at the cell surface and share a common structure composed of an extracellular ligand-binding domain, transmembrane segment, and an intracellular tyrosine kinase domain [114]. LS-174T colorectal carcinoma cells exposed to PGE_2 show rapid induction of Akt signaling. EGFR-specific tyrosine kinase inhibitors can completely abolish Akt activation. This rapid transactivation of the EGFR occurs via an intracellular pathway because inactivation of EGFR ligands with inhibitory antibodies did not inhibit PGE_2-mediated Akt activation (Fig. 2) [116]. In Caco-2, LoVo and HT-29 colon cancer cell lines PGE_2 exposure induces phosphorylation and therefore activation of downstream targets of the EGFR pathway such as ERK2. Inactivation of EGFR kinase with selective inhibitors significantly reduces PGE_2-induced ERK2 activation [117]. PGE_2, however, can also directly activate EGFR signaling and thereby stimulate cell proliferation [116,118]. The mechanism by which this occurs includes PGE_2-mediated metalloproteinase activation resulting in shedding of EGFR ligand from the plasma membrane and thus enhanced EGFR signaling. Another mechanism involves activation of the cAMP/protein kinase A pathway leading to increased expression of amphiregulin, a ligand of EGFR [119].

The TNF family of death receptors and ligands is a family of apoptosis-inducing proteins. Several human death receptors have been identified [120,121]. Apoptosis is triggered upon binding of specific TNF superfamily ligands, such as TNF, FasL (CD95L/APO-1L) or TNF-related apoptosis-inducing ligand (TRAIL), to a receptor TNFR1, TNFR2, Fas or DR4 (TRAIL-R1/APO-2)/DR5 (TRAIL-R2/KILLER/TRICK2), respectively. Besides binding to the agonistic receptors TNFR1, TNFR2, Fas, DR4 and DR5, FasL can also bind to the soluble inhibitory decoy receptor, DcR3, while TRAIL can bind to membrane-bound DcR1 (TRAIL-R3) and DcR2 (TRAIL-R4), which both lack functional death domains and are therefore unable to induce apoptosis. TRAIL can also bind to a soluble TNF family receptor, osteoprotegerin. However, the physiological significance of this interaction appears to be minimal. Upon trimerization of the death receptors, an intracellular death-inducing signaling complex (DISC) is formed composed of trimerized receptor molecules and recruited TNF receptor associated death domain (TRADD) or Fas-associated death domain (FADD) and procaspase-8 molecules. Following DISC assembly a cascade of effector caspases and substrates are activated. The nucleus is condensed and fragmented, the cytoplasm is decreased and apoptosis induction is complete [122,123].

Several studies revealed that death receptor apoptosis pathways are involved in chemotherapy-induced apoptosis. NSAIDs are also capable of inducing apoptosis via the TRAIL and Fas signaling pathways as was described by Han et al. They showed that FADD is necessary to induce apoptosis with indomethacin in Jurkat cells [124]. Indomethacin also induces apoptosis in an acquired doxorubicin resistant SCLC cell line via death receptor signaling [125]. In a hepatocellular cell line model, exposure to NS-398 resulted in Fas ligand upregulation and Fas-mediated apoptosis in COX-2 overexpressing cell lines [126]. TRAIL receptor DR5 was upregulated in colon, prostate and NSCLC cell lines after exposure to sulindac sulfide and celecoxib, respectively. These NSAIDs induced apoptosis, which was further enhanced in combination with TRAIL [127,128] (Fig. 3). COX-2 inhibition can be directly involved in enhancing TRAIL-mediated apoptosis as demonstrated in HT29 colon cancer cells. Both COX-2 inhibition and COX-2 downregulation induced clustering of DR5 at the cell surface and redistribution of the death-inducing signaling complex components (DR5, FADD, and procaspase-8) into cholesterol-rich and ceramide-rich domains [129]. Besides the upregulation of DR5, NSAIDs in particular sulindac might also enhance TRAIL-sensitivity by down-regulating survivin expression [130–132]. Other downstream components of the apoptosis pathway such as anti-apoptotic members of the Bcl-2 family can be affected by NSAIDs as well. For example, the reduction in expression of the anti-apoptotic protein Bcl-X_L in Bax-proficient HCT116 cells by sulindac sulfide augmented TRAIL induced apoptosis. The effect of sulindac sulfide on Bcl-X_L is probably due to a reduction in NF-κB activity [133].

5.1.3. Combination of NSAIDs and chemotherapy in cancer animal models

Teicher et al. studied the effect of various different chemotherapeutic agents in combination with NSAIDs on Lewis lung carcinoma bearing mice. Sulindac was an effective modulator of the chemotherapeutic agents cisplatin, cyclophosphamide, melphalan, and carmustine [134]. Indomethacin, doxorubicin and cisplatin only partially reduced the growth of colon tumors inoculated into mice after treatment with the individual drugs. However, a marked synergistic effect was observed when the combinations of indomethacin/bleomycin, indomethacin/doxorubicin or indomethacin/cisplatin were given [135,136]. Rofecoxib treatment of BALB/c mice inoculated with MC-26 colorectal cancer cells increased survival of the mice and decreased metastatic potential of the cancer cells [137]. In combination with IL-2, indomethacin could eradicate melanoma lung metastases in a nude mice model by stimulating natural killer cell formation [138]. In a dog model, the effect of cisplatin could be positively modulated with a NSAID. Dogs with naturally occurring transitional cell carcinomas of the bladder received cisplatin alone or combined with the non-specific NSAID piroxicam. About 10 of 14 dogs in the piroxicam cisplatin group showed complete or partial remission whereas none out of 8 dogs showed remission in the cisplatin group [139].

Table 1
Prognosis of patients with COX-2 overexpressing tumors compared to patients with non-overexpressing tumors

Author	Tumor type	Number of patients	Method	Decreased survival
Denkert [178]/Ristimaki [179]	Breast cancer	221/1576	IHC	Yes
Wulfing [180]	Breast cancer	200	IHC	No
Yuan [181]	Non-small cell lung cancer	60	IHC, RT-PCR	Yes
Kim [182]	Non-small cell lung cancer	84	IHC	Yes
Khuri [161]	Non-small cell lung cancer	160	ISH	Yes
Ferrandina [183]	Cervical carcinoma	175	IHC	Yes
Chen [184]	Cervical adenocarcinoma	53	IHC	No
Erkinheimo [185]	Ovarian serous carcinoma	442	IHC, RT-PCR, WB	Yes
Gallo [186]	Head and neck squamous cell carcinoma	52	IHC	Yes
Ranelletti [187]	Oropharyngeal squamous cell carcinoma	61	IHC	No
Wulfing [188]/Kim [189]	Bladder cancer	157/37	IHC	Yes
Soumaoro [190]	Colorectal cancer	288	IHC	Yes
Hull [191]	Colorectal cancer metastases	35	IHC	No
Baldi [192]/Edwards [193]	Mesothelioma	29/48	IHC	Yes
Buskens [194]	Esophageal adenocarcinoma	145	IHC	Yes
Okano [195]	Gastric cancer	166	IHC	Yes

IHC: immunohistochemistry; RT-PCR: reverse transcriptase PCR; WB: Western blot; ISH: *in situ* hybridization.

6. COX-2 expression in human cancer

6.1. COX-2 expression in normal and malignant tissues

COX-2 is constitutively expressed in the human brain, testis, kidney and central nervous system as well as in premalignant and malignant lesions. COX-2 expression can be rapidly upregulated in macrophages, synoviocytes, fibroblasts, osteoblasts, tumor endothelial cells and "activated" endothelial cells [140]. COX-2 expression is described in several tumor types including, colorectal, gastric, esophageal, hepatocellular, pancreatic, head and neck, non-small cell lung, ovarian, breast, bladder, cervical, endometrial and skin cancer [141–164]. COX-2 expression is also present in several types of premalignant lesions such as, colorectal adenomas, gastric intestinal metaplasia, Barrett's esophagus, chronic hepatitis, oral leucoplakia, atypical adenomatous hyperplasia of the lung, ductal carcinoma *in situ* of the breast, prostatic intraepithelial neoplasia, bladder dysplasia, cervical dysplasia, actinic keratoses [165–171]. COX-2 expression is not only increased in premalignant and malignant lesions, it can, e.g. also be increased in the tissue surrounding a malignant lesion [160]. In most studies, COX-2 expression in tumors or premalignant lesions was compared with surrounding tissues and not with other tumor types, which makes a comparison of COX-2 expression between different tumor types difficult. In gastric cancer patients, COX-2 expression is higher in tumors from advanced stage patients and in tumors from patients with lymph node metastases compared to patients with smaller tumors [172,173]. However, there are also studies in gastric cancer patients addressing lymphatic invasion, disease stage, or recurrence rate that do not show a correlation between COX-2 expression and more advanced disease [174]. In colorectal cancer a higher COX-2 tumor expression correlated with larger tumor size, deeper invasion and lymph node metastases [175–177]. However, the therapeutic significance of differences in COX-2 expression is questionable, since the anti-cancer effect of NSAIDs may well be COX-2 independent, which needs to be further investigated in clinical studies.

6.2. COX-2 expression and prognosis

In a number of tumors COX-2 overexpression is associated with a worse time to progression or overall survival. In the present review, we have, however, only included prospective studies or studies investigating consecutive cases, as have been described for breast cancer, non-small cell lung, ovarian, esophageal, bladder, gastric cancer as well as malignant mesothelioma [178–195]. In Table 1 survival prognosis of patients with COX-2 overexpressing tumors was compared with patients that did not have a COX-2 overexpressing tumor. In certain tumor types COX-2 overexpression is also associated with other tumor features, which themselves are predictive of worse survival such as Her-2 overexpression in breast cancer [179] and increased VEGF production in the stromal cells surrounding NSCLCs [181]. There are also tumor types in which no correlation between COX-2 overexpression and a more malignant phenotype has been observed. These cancers include colorectal, cervical, head and neck. For some other tumor types contradictionary results are reported [180,184,187,191] (Table 1). In these tumor types the importance of COX-2 in carcinogenesis or tumor progression remains questionable.

7. NSAIDs and cancer treatment

7.1. NSAIDs in clinical studies and ongoing trials in cancer patients

A number of clinical studies with NSAIDs in cancer patients are available or ongoing. NDAIDs have been investi-

gated in different types of tumors or tumor stages, and different combinations with chemotherapy. Although the focus of clinical oncological research with NSAIDs was on chemoprevention, the last years the potential therapeutic use of NSAIDs in cancer also obtained attention [196]. The preventive use of selective COX-2 inhibitors was extensively investigated in persons carrying the APC mutation that results in the familial adenomatous polyposis phenotype. The potential therapeutic use of NSAIDs is currently being investigated in several other tumor types.

First, the clinical studies investigating NSAIDs as monotherapy will be discussed. In the early 1990s, indomethacin was already investigated as an anti-cancer agent in a randomized trial conducted in 135 patients with advanced stage cancer (mainly colorectal cancer and liver, pancreatic, and gastric primary cancers) and an expected survival of more than 6 months. At that time less chemotherapy options were available. The patients were randomized to receive placebo, prednisolone, or indomethacin until death. The addition of indomethacin prolonged mean survival with 8.7 months compared to placebo-treated patients [197]. In a pilot study 12 patients, who had biochemical relapse of their prostate cancer after radiotherapy or radical prostatectomy received celecoxib. Prostate specific antigen (PSA) doubling times were calculated. Five patients had a decline in their absolute PSA level, three patients had stabilization of the level and of the remaining four patients, three had a marked decrease in their PSA doubling time [198]. In 14 cervical cancer patients tumor material was evaluated at baseline and after 10 days of celecoxib treatment. Celecoxib treatment decreases tumor COX-2 expression and markers of proliferation and neoangiogenesis [199]. In a randomized placebo controlled trial in colorectal cancer patients scheduled for resection of liver metastases 23 patients received rofecoxib and 21 patients received placebo prior to surgery. Only marginally histologically differences were observed in resected metastases of rofecoxib treated patients, without differences in apoptosis index or proliferation index [200]. At the Annual Meeting of the American Association for Cancer Research 2006, phase III results with celecoxib were presented. Fewer polyps were observed in patients with sporadic adenomas taking celecoxib compared to patients taking placebo. This effect was observed in the Adenoma Prevention with Celecoxib (APC) trial and the Prevention of Colorectal Sporadic Adenomatous Polyps (PreSAP) trial, both double blind randomized controlled trials investigating patients that have undergone removal of colorectal polyps. However, an increase in cardiovascular events was seen in both studies in the celecoxib group compared to the placebo group [201,202].

In addition to the anti-tumor activity of NSAIDs as single agents, there is interest in the effects of a combined therapy of chemotherapy with NSAIDs. In a small retrospective study comparing capecitabine in combination with celecoxib compared to capecitabine alone in colorectal cancer patients, the tumor response was increased in the capecitabine/celecoxib group compared to the capecitabine group alone as measured by, proportion of stable disease (62.5% versus 22.8%, $P = 0.001$), and increase in median time to tumor progression (6 months versus 3 months, $P = 0.002$). This effect was seen despite the fact that patients on capecitabine/celecoxib had less favorable disease characteristics (age, performance status, and prior chemotherapies) [203]. In a case report, a small cell lung carcinoma patient was described with multiple brain, lung, liver, and bone metastases recurrence after intensive chemotherapy. The patient showed no signs of remission following cisplatin, etoposide, cyclophosphamide and vincristine chemotherapy. One cycle of vincristine, methotrexate and indomethacin resulted in signs of almost complete remission without any obvious adverse effects. The patient however did not receive methotrexate in the first cycle of chemotherapy [204]. In a phase II trial, 29 patients with stages IB–IIIA NSCLC were treated with preoperative celecoxib daily in combination with two cycles of paclitaxel and carboplatin. Twenty-six patients completed preoperative celecoxib treatment and 28 patients underwent complete resection of their tumors. There were no complete pathologic responses, seven patients had minimal residual disease [205]. In a very small study investigating neoadjuvant anti-aromatase therapy (exemestane and letrozole) and celecoxib, 20 patients were randomly assigned to exemestane and celecoxib, exemestane or letrozole. All groups showed a decrease in tumor area. However, the differences between the three groups were not significant [206]. In HER-2/neu-overexpressing metastatic breast cancer patients that had progressed while receiving trastuzumab, the addition of celecoxib did not induce objective responses [207]. Rofecoxib in combination with 5-FU and leucovorin did not increase tumor response in colorectal cancer patients. After evaluating the first 10 patients that had entered the study, the study was terminated [208]. Another approach to treat metastatic cancer is targetting endothelial cells in order to inhibit tumor angiogenesis. Twelve patients with pretreated advanced melanoma received treosulfan chemotherapy in combination with rofecoxib. In one patient a partial response occurred and four showed stabilization of their disease [209]. In a prospective multicenter EORTC phase III study 85 patients were randomized to receive either the FOLFIRI regimen or the CAPE/IRI regimen. Both groups were also randomized to receive 2 mg × 400 mg celecoxib or placebo. This trial was originally designed to include 692 patients, but only 85 patients were randomized due to occurrence of 8 fatal events in this study probably caused by the chemotherapeutic agents and not by celecoxib [210]. Regretfully all the studies mentioned above are small and underpowered and do not allow firm conclusions. Currently a number of phases I–III clinical trials are still investigating the efficacy of selective COX-2 inhibitors as single agent or in combination with chemotherapy in cancer therapy (Table 2).

Table 2
Current open clinical trials with selective COX-2 inhibitors extracted from NCI's physician data query

Cancer	Phase	Projected accrual	Tumor stage	Drug
Prostate	II	66	D3	Celecoxib
Prostate	II	28	D3	Celecoxib
Prostate	II/III	3300	High risk newly diagnosed disease	Celecoxib
Prostate	II	70	D3	Celecoxib
NSCLC	II	110	IIIA	Celecoxib
NSCLC	I	24	IIIB/IV	Celecoxib
NSCLC	II	80	II/III	Celecoxib
NSCLC	I	6–45	IIB/IV	Celecoxib
Pulmonal or pleural malignancies	I	40	Inoperable disease	Celecoxib
Esophageal	I	25	Neoadjuvant	Celecoxib
Gastric/gastroesophageal junction carcinoma	II	20	Unresectable, recurrent, or metastatic	Celecoxib
Pancreas	I	20	I/II	Celecoxib
Breast	II	34	II–IV	Celecoxib
Breast/colorectal	III	342	IV/IV	Celecoxib
Colorectal	I/II	80	Metastatic colorectal carcinoma	Celecoxib
Colorectal	II	Not specified	Metastatic colorectal cancer or local recurrence	Celecoxib
Rectal	II	19	II/III	Celecoxib
Rectal	I/II	39	III/IV	Celecoxib
Glioblastoma multiforme	II	176	Adjuvant treatment	Celecoxib
Ewing sarcoma	II	6–36	Metastatic disease	Celecoxib
Advanced solid tumors	I	66	Locally advanced or metastatic disease	Celecoxib

7.2. NSAIDs to decrease chemotherapy associated side effects in cancer patients

NSAIDs can also decrease chemotherapy associated side effects. In 67 patients with metastatic colorectal cancer, side effects, such as diarrhea and the hand–foot syndrome, were decreased using capecitabine in combination with celecoxib compared to patients using capecitabine alone [211]. A combination of increased efficacy and decreased chemotherapy-induced side effects can thus be envisioned. Celecoxib increases CPT-11 cytotoxicity in colorectal cancer xenograft mouse models (HT29 cells and Colon-26 cells in nude mice and BALB/c mice), but it can also decrease the severity of CPT-induced late diarrhea. The reduction of diarrhea by selective COX-2 inhibitor suggests an inflammatory component in the pathogenesis of CPT-11-induced late diarrhea. Trifan et al. have shown that COX-2 is induced in the rat colon after CPT-11 treatment and that this is concurrent with an increase in PGE_2 production [212].

7.3. Safety profile of NSAIDs in cancer treatment

Conventional NSAIDs are well-known for their nephrotoxicity. These adverse renal effects occur because of decreased PGs produced by cyclooxygenases. Animal and human data show that COX-2 synthesized prostaglandins are important in the modulation of renal physiology. Therefore, selective COX-2 inhibitors are equal in causing nephrotoxicity as the non-selective NSAIDs [213].

NSAID use increases the risk of gastric and/or duodenal mucosal injury: erosions, ulcers and ulcer complications, especially bleeding [214,215]. About 15–30% of regular NSAID users have one or more ulcers when examined endoscopically, and 3–4.5% of NSAID users have clinically significant upper gastrointestinal events, including ulcers and ulcer complications. In the VIGOR study the risk of developing a clinically important upper gastrointestinal event in rheumatoid arthritis patients receiving rofecoxib was compared to patients receiving naproxen. Rofecoxib use was associated with a lower risk of developing clinically important upper gastrointestinal events with a decrease from 3.0 to 1.4% [216,29].

However, results from recently performed large placebo controlled studies with selective COX-2 inhibitors such as rofecoxib, celecoxib and valdecoxib monotherapy suggest that the use of these agents is not without side effects. The use of rofecoxib was associated with a significant increase in cardiovascular events as was shown in the VIGOR and APPROVe study. The incidence of myocardial infarction increased from 0.1% in the control group to 0.4% in the rofecoxib group. This effect was contributed to the cardioprotective properties of naproxen. In the APPROVe study, the cardiovascular toxicity of rofecoxib was investigated in patients with a history of colorectal adenomas that were treated to reduce the risk of recurrence of neoplastic polyps of the large bowel. Cardiovascular toxicity was lower in the placebo group (0.9%) compared to the rofecoxib group (2.4%). The use of celecoxib was also associated with a significant increase in cardiovascular events (0.7% versus 0.1%) as shown in the APC study in which patients with adenomatous polyps in colon or rectum were treated with celecoxib or placebo [217]. Therefore, the potential benefit of selective COX-2 inhibitors in cancer treatment should also be carefully weighed against the increased risk of cardiovascular events. Before these considerations can be

made data from solid phase III clinical studies must become available.

8. Conclusion

In cell line models NSAIDs in general are potent anti-tumor agents. NSAIDs can inhibit angiogenesis, proliferation, invasive growth, and induce apoptosis in a COX-2 dependent or independent manner. There is a great diversity in mechanisms causing the anti-tumor effect of NSAIDs. The inhibition of PGE_2 production as well as the inhibition of transcriptional activity of COX-2 are claimed to be the key mechanisms. However, the concentrations needed to induce COX-2 independent anti-tumor effects using NSAIDs as monotherapy are mostly not clinically achievable. A major issue in the development of new treatment schedules in solid tumors is the safety profile of the selective or non-selective COX-2 inhibitors. The non-selective COX-2 inhibitors are known for their gastrointestinal toxicity and recent evidence has emerged that the selective COX-2 inhibitors increase the risk of cardiovascular events in non-cancer patients. Both risks of these adverse effects must be taken into account when selective or non-selective COX-2 inhibitors are being prescribed to cancer patients. In the preclinical phase of drug development there is accumulating evidence that chemotherapy as well as a number of experimental or novel registered drugs, e.g. trastuzumab or rhTRAIL, may have great synergistic efficacy when combined with NSAIDs. Therefore, phases II and III clinical studies have to establish the role of NSAIDs in the treatment of cancer patients.

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References

[1] Imperiale TF. Aspirin and the prevention of colorectal cancer. N Engl J Med 2003;348:879–80.
[2] Koehne CH, DuBois RN. COX-2 inhibition and colorectal cancer. Semin Oncol 2004;31:12–21.
[3] Khuder SA, Mutgi AB. Breast cancer and NSAIDs use: a metaanalysis. Br J Cancer 2001;84:1188–92.
[4] Castelao JE, Yuan JM, Gago-Dominguez M, Yu MC, Ross RK. Non steroidal anti-inflammatory drugs and bladder cancer prevention. Br J Cancer 2000;82:1364–9.
[5] Irani J, Ravery V, Pariente JL, et al. Effect of non steroidal anti-inflammatory agents and finasteride on prostate cancer risk. J Urol 2002;168:1985–8.
[6] Harris RE, Beebe-Donk J, Schuller HM. Chemoprevention of lung cancer by non-steroidal anti-inflammatory drugs among cigarette smokers. Oncol Rep 2002;9:693–5.
[7] Muscat JE, Chen SQ, Richie Jr JP, et al. Risk of lung carcinoma among users of nonsteroidal antiinflammatory drugs. Cancer 2003;97:1732–6.
[8] Lands WE. The biosynthesis and metabolism of prostaglandins. Annu Rev Physiol 1979;41:633–52.
[9] Merlie JP, Fagan D, Mudd J, Needleman P. Isolation and characterization of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxide synthase (cyclooxygenase). J Biol Chem 1988;263:3550–3.
[10] DeWitt DL, Smith WL. Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. Proc Natl Acad Sci USA 1988;85:1412–6.
[11] Yokoyama C, Takai T, Tanabe T. Primary structure of sheep prostaglandin endoperoxide synthase deduced from cDNA sequence. FEBS Lett 1988;231:347–51.
[12] Xie WL, Chipman JG, Robertson DL, Erikson RL, Simmons DL. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci USA 1991;88:2692–6.
[13] Chandrasekharan NV, Dai H, Roos KL, et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci USA 2002;15(99):13926–31.
[14] Josephy PD, Chiu AL, Eling TE. Prostaglandin H synthase-dependent mutagenic activation of benzidine in a *Salmonella typhimurium* Ames tester strain possessing elevated *N*-acetyltransferase levels. Cancer Res 1989;15(49):853–6.
[15] Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000;69: 145–82.
[16] Herschman HR. Prostaglandin synthase 2. Biochim Biophys Acta 1996;5(1299):125–40.
[17] Dubois RN, Abramson SB, Crofford L, et al. Cyclooxygenase in biology and disease. FASEB J 1998;12:1063–73.
[18] Herschman HR, Xie W, Reddy S. Inflammation, reproduction, cancer and all that . . .. The regulation and role of the inducible prostaglandin synthase. Bioessays 1995;17:1031–7.
[19] Kage K, Fujita N, Oh-hara T, Ogata E, Fujita T, Tsuruo T. Basic fibroblast growth factor induces cyclooxygenase-2 expression in endothelial cells derived from bone. Biochem Biophys Res Commun 1999;254:259–63.
[20] Fong CY, Pang L, Holland E, Knox AJ. TGF-beta1 stimulates IL-8 release, COX-2 expression, and PGE(2) release in human airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 2000;279:L201–7.
[21] Saha D, Datta PK, Sheng H, et al. Synergistic induction of cyclooxygenase-2 by transforming growth factor-beta1 and epidermal growth factor inhibits apoptosis in epithelial cells. Neoplasia 1999;1:508–17.
[22] Diaz A, Chepenik KP, Korn JH, Reginato AM, Jimenez SA. Differential regulation of cyclooxygenases 1 and 2 by interleukin-1 beta, tumor necrosis factor-alpha, and transforming growth factor-beta 1 in human lung fibroblasts. Exp Cell Res 1998;241:222–9.
[23] Kosaka T, Miyata A, Ihara H, et al. Characterization of the human gene (PTGS2) encoding prostaglandin-endoperoxide synthase 2. Eur J Biochem 1994;221:889–97.
[24] Tanabe T, Tohnai N. Cyclooxygenase isozymes and their gene structures and expression. Prostaglandins Other Lipid Mediat 2002;68–69:95–114.
[25] Iniguez MA, Martinez-Martinez S, Punzon C, Redondo JM, Fresno M. An essential role of the nuclear factor of activated T cells in the

regulation of the expression of the cyclooxygenase-2 gene in human T lymphocytes. J Biol Chem 2000;275:23627–35.
[26] Liu Y, Borchert GL, Phang JM. Polyoma enhancer activator 3, an ets transcription factor, mediates the induction of cyclooxygenase-2 by nitric oxide in colorectal cancer cells. J Biol Chem 2004;279:18694–700.
[27] Miller C, Zhang M, He Y, et al. Transcriptional induction of cyclooxygenase-2 gene by okadaic acid inhibition of phosphatase activity in human chondrocytes: co-stimulation of AP-1 and CRE nuclear binding proteins. J Cell Biochem 1998;69:392–413.
[28] Morham SG, Langenbach R, Loftin CD, et al. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. Cell 1995;83:473–82.
[29] Bombardier C, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. N Engl J Med 2000;343:1520–8.
[30] Nussmeier NA, Whelton AA, Brown MT, et al. Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. N Engl J Med 2005;352:1081–91.
[31] Silverstein FE, Faich G, Goldstein JL, et al. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: a randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. JAMA 2000;284:1247–55.
[32] Bjorkman DJ. The effect of aspirin and nonsteroidal anti-inflammatory drugs on prostaglandins. Am J Med 1998;105: 8S–12S.
[33] Kato M, Nishida S, Kitasato H, Sakata N, Kawai S. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of non-steroidal anti-inflammatory drugs: investigation using human peripheral monocytes. J Pharm Pharmacol 2001;53:1679–85.
[34] Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. Proc Natl Acad Sci USA 1999;96: 7563–8.
[35] Patrignani P, Panara MR, Sciulli MG, Santini G, Renda G, Patrono C. Differential inhibition of human prostaglandin endoperoxide synthase-1 and -2 by nonsteroidal anti-inflammatory drugs. J Physiol Pharmacol 1997;48:623–31.
[36] Arico S, Pattingre S, Bauvy C, et al. Celecoxib induces apoptosis by inhibiting 3-phosphoinositide-dependent protein kinase-1 activity in the human colon cancer HT-29 cell line. J Biol Chem 2002;277:27613–21.
[37] Dannenberg AJ, Altorki NK, Boyle JO, et al. Cyclo-oxygenase 2: a pharmacological target for the prevention of cancer. Lancet Oncol 2001;2:544–51.
[38] Wang D, Dubois RN. Prostaglandins and cancer. Gut 2006; 55:115–22.
[39] Hull MA. Cyclooxygenase-2: how good is it as a target for cancer chemoprevention? Eur J Cancer 2005;41:1854–63.
[40] Oshima M, Oshima H, Kitagawa K, Kobayashi M, Itakura C, Taketo M. Loss of Apc heterozygosity and abnormal tissue building in nascent intestinal polyps in mice carrying a truncated Apc gene. Proc Natl Acad Sci USA 1995;92:4482–6.
[41] Oshima M, Dinchuk JE, Kargman SL, et al. Suppression of intestinal polyposis in Apc delta716 knock-out mice by inhibition of cyclooxygenase 2 (COX-2). Cell 1996;87:803–9.
[42] Moser AR, Pitot HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 1990;247:322–4.
[43] Shoemaker AR, Gould KA, Luongo C, Moser AR, Dove WF. Studies of neoplasia in the Min mouse. Biochim Biophys Acta 1997;1332:F25–48.
[44] Chulada PC, Thompson MB, Mahler JF, et al. Genetic disruption of Ptgs-1, as well as Ptgs-2, reduces intestinal tumorigenesis in Min mice. Cancer Res 2000;60:4705–8.
[45] Liu CH, Chang SH, Narko K, et al. Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. J Biol Chem 2001;276:18563–9.
[46] Seed MP, Brown JR, Freemantle CN, et al. The inhibition of colon-26 adenocarcinoma development and angiogenesis by topical diclofenac in 2.5% hyaluronan. Cancer Res 1997;57:1625–9.
[47] Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669–76.
[48] Kuwashima L, Graeber J, Glaser BM. Stimulation of endothelial cell prostacyclin release by retina-derived factors. Invest Ophthalmol Vis Sci 1988;29:1213–20.
[49] Murohara T, Horowitz JR, Silver M, et al. Vascular endothelial growth factor/vascular permeability factor enhances vascular permeability via nitric oxide and prostacyclin. Circulation 1998;97:99–107.
[50] Liu XH, Kirschenbaum A, Lu M, et al. Prostaglandin E2 induces hypoxia-inducible factor-1alpha stabilization and nuclear localization in a human prostate cancer cell line. J Biol Chem 2002;277:50081–6.
[51] Krysan K, Reckamp KL, Dalwadi H, et al. Prostaglandin E2 activates mitogen-activated protein kinase/Erk pathway signaling and cell proliferation in non-small cell lung cancer cells in an epidermal growth factor receptor-independent manner. Cancer Res 2005;65:6275–81.
[52] Cheng T, Cao W, Wen R, Steinberg RH, LaVail MM. Prostaglandin E2 induces vascular endothelial growth factor and basic fibroblast growth factor mRNA expression in cultured rat Muller cells. Invest Ophthalmol Vis Sci 1998;39:581–91.
[53] Amano H, Hayashi I, Endo H, et al. Host prostaglandin E(2)-EP3 signaling regulates tumor-associated angiogenesis and tumor growth. J Exp Med 2003;197:221–32.
[54] Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 1998;93:705–16.
[55] Williams CS, Tsujii M, Reese J, Dey SK, DuBois RN. Host cyclooxygenase-2 modulates carcinoma growth. J Clin Invest 2000;105:1589–94.
[56] Lim SC. Role of COX-2, VEGF and cyclin D1 in mammary infiltrating duct carcinoma. Oncol Rep 2003;10:1241–9.
[57] Timoshenko AV, Chakraborty C, Wagner GF, Lala PK. COX-2-mediated stimulation of the lymphangiogenic factor VEGF-C in human breast cancer. Br J Cancer 2006;94(8):1154–63.
[58] Masferrer JL, Leahy KM, Koki AT, et al. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 2000;60:1306–11.
[59] Brueggemeier RW, Quinn AL, Parrett ML, Joarder FS, Harris RE, Robertson FM. Correlation of aromatase and cyclooxygenase gene expression in human breast cancer specimens. Cancer Lett 1999;140:27–35.
[60] Karuppu D, Kalus A, Simpson ER, Clyne C. Aromatase and prostaglandin inter-relationships in breast adipose tissue: significance for breast cancer development. Breast Cancer Res Treat 2002;76:103–9.
[61] Sheng H, Shao J, Washington MK, DuBois RN. Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. J Biol Chem 2001;276:18075–81.
[62] 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.
[63] Singh B, Berry JA, Shoher A, Ramakrishnan V, Lucci A. COX-2 overexpression increases motility and invasion of breast cancer cells. Int J Oncol 2005;26:1393–9.
[64] Davis TW, O'Neal JM, Pagel MD, et al. Synergy between celecoxib and radiotherapy results from inhibition of cyclooxygenase-2-derived prostaglandin E2, a survival factor for tumor and associated vasculature. Cancer Res 2004;64:279–85.
[65] Terakado N, Shintani S, Yano J, et al. Overexpression of cyclooxygenase-2 is associated with radioresistance in oral squamous cell carcinoma. Oral Oncol 2004;40:383–9.

[66] Shin YK, Park JS, Kim HS, et al. Radiosensitivity enhancement by celecoxib, a cyclooxygenase (COX)-2 selective inhibitor, via COX-2-dependent cell cycle regulation on human cancer cells expressing differential COX-2 levels. Cancer Res 2005;65:9501–9.
[67] Reed JC. Dysregulation of apoptosis in cancer. J Clin Oncol 1999;17:2941–53.
[68] Campos L, Rouault JP, Sabido O, et al. High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. Blood 1993;81:3091–6.
[69] Bargou RC, Daniel PT, Mapara MY, et al. Expression of the bcl-2 gene family in normal and malignant breast tissue: low bax-alpha expression in tumor cells correlates with resistance towards apoptosis. Int J Cancer 1995;60:854–9.
[70] Prokop A, Wieder T, Sturm I, et al. Relapse in childhood acute lymphoblastic leukemia is associated with a decrease of the Bax/Bcl-2 ratio and loss of spontaneous caspase-3 processing in vivo. Leukemia 2000;14:1606–13.
[71] Sturm I, Kohne CH, Wol G, et al. Analysis of the p53/BAX pathway in colorectal cancer: low BAX is a negative prognostic factor in patients with resected liver metastases. J Clin Oncol 1999;17:1364–74.
[72] Lima RT, Martins LM, Guimaraes JE, Sambade C, Vasconcelos MH. Specific downregulation of bcl-2 and xIAP by RNAi enhances the effects of chemotherapeutic agents in MCF-7 human breast cancer cells. Cancer Gene Ther 2004;11:309–16.
[73] Zangemeister-Wittke U. Antisense to apoptosis inhibitors facilitates chemotherapy and TRAIL-induced death signaling. Ann N Y Acad Sci 2003;1002:90–4.
[74] Nita ME, Nagawa H, Tominaga O, et al. 5-Fluorouracil induces apoptosis in human colon cancer cell lines with modulation of Bcl-2 family proteins. Br J Cancer 1998;78:986–92.
[75] Liu XH, Yao S, Kirschenbaum A, Levine AC. NS398, a selective cyclooxygenase-2 inhibitor, induces apoptosis and down-regulates bcl-2 expression in LNCaP cells. Cancer Res 1998;58:4245–9.
[76] Zhang L, Yu J, Park BH, Kinzler KW, Vogelstein B. Role of BAX in the apoptotic response to anticancer agents. Science 2000;290:989–92.
[77] Zhou XM, Wong BC, Fan XM, et al. Non-steroidal anti-inflammatory drugs induce apoptosis in gastric cancer cells through up-regulation of bax and bak. Carcinogenesis 2001;22:1393–7.
[78] Ding H, Han C, Zhu J, Chen CS, D'Ambrosio SM. Celecoxib derivatives induce apoptosis via the disruption of mitochondrial membrane potential and activation of caspase 9. Int J Cancer 2005;113:803–10.
[79] Narayanan BA, Condon MS, Bosland MC, Narayanan NK, Reddy BS. Suppression of *N*-methyl-*N*-nitrosourea/testosterone-induced rat prostate cancer growth by celecoxib: effects on cyclooxygenase-2, cell cycle regulation, and apoptosis mechanism(s). Clin Cancer Res 2003;9:3503–13.
[80] Gajewski TF, Thompson CB. Apoptosis meets signal transduction: elimination of a BAD influence. Cell 1996;87:589–92.
[81] Cardone MH, Roy N, Stennicke HR, et al. Regulation of cell death protease caspase-9 by phosphorylation. Science 1998;282:1318–21.
[82] Hsu AL, Ching TT, Wang DS, Song X, Rangnekar VM, Chen CS. The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. J Biol Chem 2000;275:11397–403.
[83] Basu GD, Pathangey LB, Tinder TL, Lagioia M, Gendler SJ, Mukherjee P. Cyclooxygenase-2 inhibitor induces apoptosis in breast cancer cells in an in vivo model of spontaneous metastatic breast cancer. Mol Cancer Res 2004;2:632–42.
[84] Zhang Z, Lai GH, Sirica AE. Celecoxib-induced apoptosis in rat cholangiocarcinoma cells mediated by Akt inactivation and Bax translocation. Hepatology 2004;39:1028–37.
[85] Wu T, Leng J, Han C, Demetris AJ. The cyclooxygenase-2 inhibitor celecoxib blocks phosphorylation of Akt and induces apoptosis in human cholangiocarcinoma cells. Mol Cancer Ther 2004;3:299–307.
[86] Lin MT, Lee RC, Yang PC, Ho FM, Kuo ML. Cyclooxygenase-2 inducing Mcl-1-dependent survival mechanism in human lung adenocarcinoma CL1.0 cells. Involvement of phosphatidylinositol 3-kinase/Akt pathway. J Biol Chem 2001;276:48997–9002.
[87] Adida C, Recher C, Raffoux E, et al. Expression and prognostic significance of survivin in de novo acute myeloid leukaemia. Br J Haematol 2000;111:196–203.
[88] Adida C, Berrebi D, Peuchmaur M, Reyes-Mugica M, Altieri DC. Anti-apoptosis gene, survivin, and prognosis of neuroblastoma. Lancet 1998;351:882–3.
[89] Li J, Feng Q, Kim JM, et al. Human ovarian cancer and cisplatin resistance: possible role of inhibitor of apoptosis proteins. Endocrinology 2001;142:370–80.
[90] Datta R, Oki E, Endo K, Biedermann V, Ren J, Kufe D. XIAP regulates DNA damage-induced apoptosis downstream of caspase-9 cleavage. J Biol Chem 2000;275:31733–8.
[91] Nishihara H, Kizaka-Kondoh S, Insel PA, Eckmann L. Inhibition of apoptosis in normal and transformed intestinal epithelial cells by cAMP through induction of inhibitor of apoptosis protein (IAP)-2. Proc Natl Acad Sci USA 2003;100:8921–6.
[92] Krysan K, Merchant FH, Zhu L, et al. COX-2-dependent stabilization of survivin in non-small cell lung cancer. FASEB J 2004;18:206–8.
[93] Krysan K, Dalwadi H, Sharma S, Pold M, Dubinett S. Cyclooxygenase 2-dependent expression of survivin is critical for apoptosis resistance in non-small cell lung cancer. Cancer Res 2004;64:6359–62.
[94] Zhang T, Fields JZ, Ehrlich SM, Boman BM. The chemopreventive agent sulindac attenuates expression of the antiapoptotic protein survivin in colorectal carcinoma cells. J Pharmacol Exp Ther 2004;308:434–7.
[95] Shishodia S, Aggarwal BB. Guggulsterone inhibits NF-kappaB and IkappaBalpha kinase activation, suppresses expression of anti-apoptotic gene products, and enhances apoptosis. J Biol Chem 2004;279:47148–58.
[96] Wang CY, Cusack Jr JC, Liu R, Baldwin Jr AS. Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-kappaB. Nat Med 1999;5:412–7.
[97] Guo J, Verma UN, Gaynor RB, Frenkel EP, Becerra CR. Enhanced chemosensitivity to irinotecan by RNA interference-mediated down-regulation of the nuclear factor-kappaB p65 subunit. Clin Cancer Res 2004;10:3333–41.
[98] Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 1994;265:956–9.
[99] Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. Nature 1998;396:77–80.
[100] Yamamoto Y, Yin MJ, Lin KM, Gaynor RB. Sulindac inhibits activation of the NF-kappaB pathway. J Biol Chem 1999;274:27307–14.
[101] Wong BC, Jiang X, Fan XM, et al. Suppression of RelA/p65 nuclear translocation independent of IkappaB-alpha degradation by cyclooxygenase-2 inhibitor in gastric cancer. Oncogene 2003;22:1189–97.
[102] Shishodia S, Koul D, Aggarwal BB. Cyclooxygenase (COX)-2 inhibitor celecoxib abrogates TNF-induced NF-kappa B activation through inhibition of activation of I kappa B alpha kinase and Akt in human non-small cell lung carcinoma: correlation with suppression of COX-2 synthesis. J Immunol 2004;173:2011–22.
[103] Duffy CP, Elliott CJ, O'Connor RA, et al. Enhancement of chemotherapeutic drug toxicity to human tumour cells in vitro by a subset of non-steroidal anti-inflammatory drugs (NSAIDs). Eur J Cancer 1998;34:1250–9.
[104] Kang HK, Lee E, Pyo H, Lim SJ. Cyclooxygenase-independent down-regulation of multidrug resistance-associated protein-1 expression by celecoxib in human lung cancer cells. Mol Cancer Ther 2005;4:1358–63.
[105] Puhlmann U, Ziemann C, Ruedell G, et al. Impact of the cyclooxygenase system on doxorubicin-induced functional multidrug resistance 1 overexpression and doxorubicin sensitivity in acute myeloid leukemic HL-60 cells. J Pharmacol Exp Ther 2005;312:346–54.

[106] O'Connor R, Heenan M, Connolly L, Larkin A, Clynes M. Increased anti-tumour efficacy of doxorubicin when combined with sulindac in a xenograft model of an MRP-1-positive human lung cancer. Anticancer Res 2004;24:457–64.
[107] Mizutani Y, Nakanishi H, Li YN, Sato N, Kawauchi A, Miki T. Enhanced sensitivity of bladder cancer cells to cisplatin mediated cytotoxicity and apoptosis in vitro and in vivo by the selective cyclooxygenase-2 inhibitor JTE-522. J Urol 2004;172:1474–9.
[108] Lin J, Hsiao PW, Chiu TH, Chao JI. Combination of cyclooxygenase-2 inhibitors and oxaliplatin increases the growth inhibition and death in human colon cancer cells. Biochem Pharmacol 2005;70:658–67.
[109] Czembirek C, Eder-Czembirek C, Erovic BM, Turhani D, Selzer E, Thurnher D. Inhibition of cytotoxicity of cisplatin by cyclooxygenase-2 inhibitor nimesulide in head and neck cancer cell lines. Oncol Rep 2005;14:1523–6.
[110] Vadlamudi R, Mandal M, Adam L, Steinbach G, Mendelsohn J, Kumar R. Regulation of cyclooxygenase-2 pathway by HER2 receptor. Oncogene 1999;18:305–14.
[111] Subbaramaiah K, Norton L, Gerald W, Dannenberg AJ. Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer: evidence for involvement of AP-1 and PEA3. J Biol Chem 2000;277:18649–57.
[112] Wang SC, Lien HC, Xia W, et al. Binding at and transactivation of the COX-2 promoter by nuclear tyrosine kinase receptor ErbB-2. Cancer Cell 2004;6:251–61.
[113] Mann M, Sheng H, Shao J, et al. Targeting cyclooxygenase 2 and HER-2/neu pathways inhibits colorectal carcinoma growth. Gastroenterology 2001;120:1713–9.
[114] Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. EMBO J 2000;19:3159–67.
[115] Yarden Y. The EGFR family and its ligands in human cancer: signalling mechanisms and therapeutic opportunities. Eur J Cancer 2001;37(Suppl 4):S3–8.
[116] Buchanan FG, Wang D, Bargiacchi F, DuBois RN. Prostaglandin E2 regulates cell migration via the intracellular activation of the epidermal growth factor receptor. J Biol Chem 2003;278:35451–7.
[117] Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin E2 transactivates EGF receptor a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. Nat Med 2002;8:289–93.
[118] Dannenberg AJ, Lippman SM, Mann JR, Subbaramaiah K, DuBois RN. Cyclooxygenase-2 and epidermal growth factor receptor: pharmacologic targets for chemoprevention. J Clin Oncol 2005;23:254–66.
[119] Shao J, Lee SB, Guo H, Evers BM, Sheng H. Prostaglandin E2 stimulates the growth of colon cancer cells via induction of amphiregulin. Cancer Res 2003;63:5218–23.
[120] Özören N, El-Deiry WS. Cell surface death receptor signaling in normal and cancer cells. Semin Cancer Biol 2003;13:135–47.
[121] de Jong S, Timmer T, Heijenbrok FJ, de Vries EGE. Death receptor ligands, in particular TRAIL, to overcome drug resistance. Cancer Metastasis Rev 2001;20:51–6.
[122] Hengartner MO. The biochemistry of apoptosis. Nature 2000;407:770–6.
[123] Zhang Z, Lai GH, Sirica AE. Celecoxib-induced apoptosis in rat cholangiocarcinoma cells mediated by Akt inactivation and Bax translocation. Hepatology 2004;39:1028–37.
[124] Han Z, Pantazis P, Wyche JH, Kouttab N, Kidd VJ, Hendrickson EA. A Fas-associated death domain protein-dependent mechanism mediates the apoptotic action of non-steroidal anti-inflammatory drugs in the human leukemic Jurkat cell line. J Biol Chem 2001;276:38748–54.
[125] de Groot DJ, Timmer T, Spierings DC, Le TK, de Jong S, de Vries EG. Indomethacin-induced activation of the death receptor-mediated apoptosis pathway circumvents acquired doxorubicin resistance in SCLC cells. Br J Cancer 2005;92:1459–66.
[126] Cheng AS, Chan HL, Leung WK, Wong N, Johnson PJ, Sung JJ. Specific COX-2 inhibitor, NS-398, suppresses cellular proliferation and induces apoptosis in human hepatocellular carcinoma cells. Int J Oncol 2003;23:113–9.
[127] Liu X, Yue P, Zhou Z, Khuri FR, Sun SY. Death receptor regulation and celecoxib-induced apoptosis in human lung cancer cells. J Natl Cancer Inst 2004;96:1769–80.
[128] Huang Y, He Q, Hillman MJ, Rong R, Sheikh MS. Sulindac sulfide-induced apoptosis involves death receptor 5 and the caspase 8-dependent pathway in human colon and prostate cancer cells. Cancer Res 2001;61:6918–24.
[129] Martin S, Phillips DC, Szekely-Szucs K, Elghazi L, Desmots F, Houghton JA. Cyclooxygenase-2 inhibition sensitizes human colon carcinoma cells to TRAIL-induced apoptosis through clustering of DR5 and concentrating death-inducing signaling complex components into ceramide-enriched caveolae. Cancer Res 2005;65:11447–58.
[130] He Q, Luo X, Huang Y, Sheikh MS. Apo2L/TRAIL differentially modulates the apoptotic effects of sulindac and a COX-2 selective non-steroidal anti-inflammatory agent in Bax-deficient cells. Oncogene 2002;21:6032–40.
[131] Huang Y, He Q, Hillman MJ, Rong R, Sheikh MS. Sulindac sulfide-induced apoptosis involves death receptor 5 and the caspase 8-dependent pathway in human colon and prostate cancer cells. Cancer Res 2001;61:6918–24.
[132] Tang X, Sun YJ, Half E, Kuo MT, Sinicrope F. Cyclooxygenase-2 overexpression inhibits death receptor 5 expression and confers resistance to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in human colon cancer cells. Cancer Res 2002;62:4903–8.
[133] Ravi R, Bedi A. Requirement of BAX for TRAIL/Apo2L-induced apoptosis of colorectal cancers: synergism with sulindac-mediated inhibition of Bcl-x(L). Cancer Res 2002;62:1583–7.
[134] Teicher BA, Korbut TT, Menon K, Holden SA, Ara G. Cyclooxygenase and lipoxygenase inhibitors as modulators of cancer therapies. Cancer Chemother Pharmacol 1994;33:515–22.
[135] Hattori K, Matsushita R, Kimura K, Abe Y, Nakashima E. Synergistic effect of indomethacin with adriamycin and cisplatin on tumor growth. Biol Pharm Bull 2001;24:1214–7.
[136] Matsushita R, Hattori K, Hayashi K, Iizasa H, Ichimura F, Nakashima E. Synergistic effect of indomethacin and bleomycin on tumor growth produced by activating antitumor immunity. Pharm Res 2001;18:243–5.
[137] Yao M, Kargman S, Lam EC, et al. Inhibition of cyclooxygenase-2 by rofecoxib attenuates the growth and metastatic potential of colorectal carcinoma in mice. Cancer Res 2003;63:586–92.
[138] Lala PK, Elkashab M, Kerbel RS, Parhar RS. Cure of human melanoma lung metastases in nude mice with chronic indomethacin therapy combined with multiple rounds of IL-2: characteristics of killer cells generated in situ. Int Immunol 1990;2:1149–58.
[139] Knapp DW, Glickman NW, Widmer WR, et al. Cisplatin versus cisplatin combined with piroxicam in a canine model of human invasive urinary bladder cancer. Cancer Chemother Pharmacol 2000;46:221–6.
[140] Hayashi N, Yamamoto H, Hiraoka N, et al. Differential expression of cyclooxygenase-2 (COX-2) in human bile duct epithelial cells and bile duct neoplasm. Hepatology 2001;34:638–60.
[141] Howe LR, Subbaramaiah K, Brown AM, Dannenberg AJ. Cyclooxygenase-2: a target for the prevention and treatment of breast cancer. Endocr Relat Cancer 2001;8:97–114.
[142] Kagoura M, Toyoda M, Matsui C, Morohashi M. Immunohistochemical expression of cyclooxygenase-2 in skin cancers. J Cutan Pathol 2001;28:298–302.
[143] Kulkarni S, Rader JS, Zhang F, et al. Cyclooxygenase-2 is overexpressed in human cervical cancer. Clin Cancer Res 2001;7:429–34.

[144] Marrogi A, Pass HI, Khan M, Metheny-Barlow LJ, Harris CC, Gerwin BI. Human mesothelioma samples overexpress both cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (NOS2): in vitro antiproliferative effects of a COX-2 inhibitor. Cancer Res 2000;60:3696–700.
[145] Chan G, Boyle JO, Yang EK, et al. Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. Cancer Res 1999;59:991–4.
[146] Shiota G, Okubo M, Noumi T, et al. Cyclooxygenase-2 expression in hepatocellular carcinoma. Hepatogastroenterology 1999;46:407–12.
[147] Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H, Ristimaki A. Expression of cyclooxygenase-2 in human lung carcinoma. Cancer Res 1998;58:4997–5001.
[148] Tucker ON, Dannenberg AJ, Yang EK, et al. Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer. Cancer Res 1999;59:987–90.
[149] Uefuji K, Ichikura T, Mochizuki H. Expression of cyclooxygenase-2 in human gastric adenomas and adenocarcinomas. J Surg Oncol 2001;76:26–30.
[150] Zimmermann KC, Sarbia M, Weber AA, Borchard F, Gabbert HE, Schror K. Cyclooxygenase-2 expression in human esophageal carcinoma. Cancer Res 1999;59:198–204.
[151] Kondo M, Yamamoto H, Nagano H, et al. Increased expression of COX-2 in nontumor liver tissue is associated with shorter disease-free survival in patients with hepatocellular carcinoma. Clin Cancer Res 1999;5:4005–12.
[152] Mohammed SI, Knapp DW, Bostwick DG, et al. Expression of cyclooxygenase-2 (COX-2) in human invasive transitional cell carcinoma (TCC) of the urinary bladder. Cancer Res 1999;59:5647–50.
[153] Tong BJ, Tan J, Tajeda L, et al. Heightened expression of cyclooxygenase-2 and peroxisome proliferator-activated receptor-delta in human endometrial adenocarcinoma. Neoplasia 2000;2:483–90.
[154] Muller-Decker K, Reinerth G, Krieg P, et al. Prostaglandin-H-synthase isozyme expression in normal and neoplastic human skin. Int J Cancer 1999;82:648–56.
[155] Yoshimura R, Sano H, Masuda C, et al. Expression of cyclooxygenase-2 in prostate carcinoma. Cancer 2000;89:589–96.
[156] Fujita T, Matsui M, Takaku K, et al. Size- and invasion-dependent increase in cyclooxygenase 2 levels in human colorectal carcinomas. Cancer Res 1998;58:4823–6.
[157] Sheehan KM, Sheahan K, O'Donoghue DP, et al. The relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA 1999;282:1254–7.
[158] Koki AT, Masferrer JL. Celecoxib: a specific COX-2 inhibitor with anticancer properties. Cancer Contr 2002;9:28–35.
[159] Shirahama T, Arima J, Akiba S, Sakakura C. Relation between cyclooxygenase-2 expression and tumor invasiveness and patient survival in transitional cell carcinoma of the urinary bladder. Cancer 2001;92:188–93.
[160] Achiwa H, Yatabe Y, Hida T, et al. Prognostic significance of elevated cyclooxygenase 2 expression in primary, resected lung adenocarcinomas. Clin Cancer Res 1999;5:1001–5.
[161] Khuri FR, Wu H, Lee JJ, et al. Cyclooxygenase-2 overexpression is a marker of poor prognosis in stage I non-small cell lung cancer. Clin Cancer Res 2001;7:861–7.
[162] Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994;107:1183–8.
[163] Takahashi T, Kozaki K, Yatabe Y, Achiwa H, Hida T. Increased expression of COX-2 in the development of human lung cancers. J Environ Pathol Toxicol Oncol 2002;21:177–81.
[164] Hase T, Yoshimura R, Matsuyama M, et al. Cyclooxygenase-1 and -2 in human testicular tumours. Eur J Cancer 2003;39:2043–9.
[165] Morris CD, Armstrong GR, Bigley G, Green H, Attwood SE. Cyclooxygenase-2 expression in the Barrett's metaplasia–dysplasia–adenocarcinoma sequence. Am J Gastroenterol 2001;96:990–6.
[166] Wilson KT, Fu S, Ramanujam KS, Meltzer SJ. Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's esophagus and associated adenocarcinomas. Cancer Res 1998;58:2929–34.
[167] Saukkonen K, Rintahaka J, Sivula A, et al. Cyclooxygenase-2 and gastric carcinogenesis. APMIS 2003;111:915–25.
[168] Sung JJ, Leung WK, Go MY, et al. Cyclooxygenase-2 expression in *Helicobacter pylori*-associated premalignant and malignant gastric lesions. Am J Pathol 2000;157:729–35.
[169] Shirahama T. Cyclooxygenase-2 expression is up-regulated in transitional cell carcinoma and its preneoplastic lesions in the human urinary bladder. Clin Cancer Res 2000;6:2424–30.
[170] Buckman SY, Gresham A, Hale P, et al. COX-2 expression is induced by UVB exposure in human skin: implications for the development of skin cancer. Carcinogenesis 1998;19:723–9.
[171] Hosomi Y, Yokose T, Hirose Y, et al. Increased cyclooxygenase 2 (COX-2) expression occurs frequently in precursor lesions of human adenocarcinoma of the lung. Lung Cancer 2000;30:73–81.
[172] Ohno R, Yoshingaga K, Fujita T, et al. Depth of invasison parallels increased cyclooxygenase-2 levels in patients with gastric carcinoma. Cancer 2001;91:1876–81.
[173] Murata H, Kawano S, Tsuji S, et al. Cyclooxygenase-2 overexpression enhances lymphatic invasion and metastasis in human gastric carcinoma. Am J Gastroenterol 1999;94:451–5.
[174] Lim HY, Joo HJ, Choi JH, et al. Increased expression of cyclooxygenase-2 protein in human gastric carcinoma. Clin Cancer Res 2000;6:519–25.
[175] Fujita T, Matsui M, Takaku K, et al. Size- and invasion-dependent increase in cyclooxygenase 2 levels in human colorectal carcinomas. Cancer Res 1998;58:4823–6.
[176] Sheehan KM, Sheahan K, O'Donoghue DP, et al. The relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA 1999;282:1254–7.
[177] Chen WS, Wei SJ, Liu JM, Hsiao M, Kou-Lin J, Yang WK. Tumor invasiveness and liver metastasis of colon cancer cells correlated with cyclooxygenase-2 (COX-2) expression and inhibited by a COX-2-selective inhibitor, etodolac. Int J Cancer 2001;91:894–9.
[178] Denkert C, Winzer KJ, Muller BM, et al. Elevated expression of cyclooxygenase-2 is a negative prognostic factor for disease free survival and overall survival in patients with breast carcinoma. Cancer 2003;97:2978–87.
[179] Ristimaki A, Sivula A, Lundin J, et al. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. Cancer Res 2002;62:632–5.
[180] Wulfing P, Diallo R, Muller C, et al. Analysis of cyclooxygenase-2 expression in human breast cancer: high throughput tissue microarray analysis. J Cancer Res Clin Oncol 2003;129:375–82.
[181] Yuan A, Yu CJ, Shun CT, et al. Total cyclooxygenase-2 mRNA levels correlate with vascular endothelial growth factor mRNA levels, tumor angiogenesis and prognosis in non-small cell lung cancer patients. Int J Cancer 2005;115:545–55.
[182] Kim HS, Youm HR, Lee JS, Min KW, Chung JH, Park CS. Correlation between cyclooxygenase-2 and tumor angiogenesis in non-small cell lung cancer. Lung Cancer 2003;42:163–70.
[183] Ferrandina G, Ranelletti FO, Legge F, et al. Prognostic role of the ratio between cyclooxygenase-2 in tumor and stroma compartments in cervical cancer. Clin Cancer Res 2004;10:3117–23.
[184] Chen YJ, Wang LS, Wang PH, et al. High cyclooxygenase-2 expression in cervical adenocarcinomas. Gynecol Oncol 2003;88:379–85.
[185] Erkinheimo TL, Lassus H, Finne P, et al. Elevated cyclooxygenase-2 expression is associated with altered expression of p53 and SMAD4,

amplification of HER-2/neu, and poor outcome in serous ovarian carcinoma. Clin Cancer Res 2004;10:538–45.
[186] Gallo O, Masini E, Bianchi B, Bruschini L, Paglierani M, Franchi A. Prognostic significance of cyclooxygenase-2 pathway and angiogenesis in head and neck squamous cell carcinoma. Hum Pathol 2002;33:708–14.
[187] Ranelletti FO, Almadori G, Rocca B, et al. Prognostic significance of cyclooxygenase-2 in laryngeal squamous cell carcinoma. Int J Cancer 2001;95:343–9.
[188] Wulfing C, Eltze E, von Struensee D, Wulfing P, Hertle L, Piechota H. Cyclooxygenase-2 expression in bladder cancer: correlation with poor outcome after chemotherapy. Eur Urol 2004;45:46–52.
[189] Kim SI, Kwon SM, Kim YS, Hong SJ. Association of cyclooxygenase-2 expression with prognosis of stage T1 grade 3 bladder cancer. Urology 2002;60:816–21.
[190] Soumaoro LT, Uetake H, Higuchi T, Takagi Y, Enomoto M, Sugihara K. Cyclooxygenase-2 expression: a significant prognostic indicator for patients with colorectal cancer. Clin Cancer Res 2004;10:8465–71.
[191] Hull MA, Fenwick SW, Chapple KS, Scott N, Toogood GJ, Lodge JP. Cyclooxygenase-2 expression in colorectal cancer liver metastases. Clin Exp Metastasis 2000;18:21–7.
[192] Baldi A, Santini D, Vasaturo F, et al. Prognostic significance of cyclooxygenase-2 (COX-2) and expression of cell cycle inhibitors p21 and p27 in human pleural malignant mesothelioma. Thorax 2004;59:428–33.
[193] Edwards JG, Faux SP, Plummer SM, et al. Cyclooxygenase-2 expression is a novel prognostic factor in malignant mesothelioma. Clin Cancer Res 2002;8:1857–62.
[194] Buskens CJ, Van Rees BP, Sivula A, et al. Prognostic significance of elevated cyclooxygenase 2 expression in patients with adenocarcinoma of the esophagus. Gastroenterology 2002;122:1800–7.
[195] Okano H, Shinohara H, Miyamoto A, Takaori K, Tanigawa N. Concomitant overexpression of cyclooxygenase-2 in HER-2-positive on Smad4-reduced human gastric carcinomas is associated with a poor patient outcome. Clin Cancer Res 2004;10:6938–45.
[196] Phillips RK, et al. A randomised, double blind, placebo controlled study of celecoxib, a selective cyclooxygenase 2 inhibitor, on duodenal polyposis in familial adenomatous polyposis. Gut 2002;50:857–60.
[197] Lundholm K, Gelin J, Hyltander A, et al. Anti-inflammatory treatment may prolong survival in undernourished patients with metastatic solid tumors. Cancer Res 1994;54:5602–6.
[198] Pruthi RS, Derksen JE, Moore D. A pilot study of use of the cyclooxygenase-2 inhibitor celecoxib in recurrent prostate cancer after definitive radiation therapy or radical prostatectomy. BJU Int 2004;93:275–8.
[199] Ferrandina G, Ranelletti FO, Legge F, et al. Celecoxib modulates the expression of cyclooxygenase-2, Ki67, apoptosis-related marker, and microvessel density in human cervical cancer: a pilot study. Clin Cancer Res 2003;9:4324–31.
[200] Fenwick SW, Toogood GJ, Lodge JP, Hull MA. The effect of the selective cyclooxygenase-2 inhibitor rofecoxib on human colorectal cancer liver metastases. Gastroenterology 2003;125:716–29.
[201] Arber N. Chemoprevention of colorectal adenomas with celecoxib in an international randomized, placebo-controlled, double-blind trial. American Association for Cancer Research, abstract number CP-4; 2006.
[202] Bertagnolli MM, Eagle CJ, Hawk ET, The Adenoma Prevention with Celecoxib (APC) Study. Celecoxib reduces sporadic colorectal adenomas: results from the Adenoma Prevention with Celecoxib (APC) trial. American Association for Cancer Research, abstract number CP-3; 2006.
[203] Lin E, Morris JS, Ayers GD. Effect of celecoxib on capecitabine induced hand–foot syndrome and antitumor activity. Oncology (Huntingt) 2002;16:31–7.
[204] Kobayashi S, Okada S, Hasumi T, Sato N, Fujimura S. The marked anticancer effect of combined VCR, MTX, and indomethacin against drug-resistant recurrent small cell lung carcinoma after conventional chemotherapy: report of a case. Surg Today 1999;29:666–9.
[205] Altorki NK, Keresztes RS, Port JL, et al. Celecoxib a selective cyclooxygenase-2 inhibitor, enhances the response to preoperative paclitaxel and carboplatin in early-stage non-small-cell lung cancer. J Clin Oncol 2003;21:2645–50.
[206] Chow LW, Wong JL, Toi M. Celecoxib anti-aromatase neoadjuvant (CAAN) trial for locally advanced breast cancer: preliminary report. J Steroid Biochem Mol Biol 2003;86:443–7.
[207] Dang CT, Dannenberg AJ, Subbaramaiah K, et al. Phase II study of celecoxib and trastuzumab in metastatic breast cancer patients who have progressed after prior trastuzumab-based treatments. Clin Cancer Res 2004;10:4062–7.
[208] Becerra CR, Frenkel EP, Ashfaq R, Gaynor RB. Increased toxicity and lack of efficacy of rofecoxib in combination with chemotherapy for treatment of metastatic colorectal cancer: a phase II study. Int J Cancer 2003;105:868–72.
[209] Spieth K, Kaufmann R, Gille J. Metronomic oral low-dose treosulfan chemotherapy combined with cyclooxygenase-2 inhibitor in pretreated advanced melanoma: a pilot study. Cancer Chemother Pharmacol 2003;52:377–82.
[210] Kohne C, De Greve J, Bokemeyer C, et al. Capecitabine plus irinotecan versus 5-FU/FA/irinotecan ± celecoxib in first line treatment of metastatic colorectal cancer. Safety results of the prospective multicenter EORTC phase III study 40015. J Clin Oncol 2005;23(Suppl 16):A-3252–525.
[211] Lin E, Morris JS, Ayers GD. Effect of celecoxib on capecitabine-induced hand–foot syndrome and antitumor activity. Oncology (Williston Park) 2002;16:31–7.
[212] Trifan OC, Durham WF, Salazar VS, et al. Cyclooxygenase-2 inhibition with celecoxib enhances antitumor efficacy and reduces diarrhea side effect of CPT-11. Cancer Res 2002;62:5778–84.
[213] Gambaro G, Perazella MA. Adverse renal effects of anti-inflammatory agents: evaluation of selective and nonselective cyclooxygenase inhibitors. J Intern Med 2003;253:643–52.
[214] Laine L. Approaches to nonsteroidal anti-inflammatory drug use in the high-risk patient. Gastroenterology 2001;120:594–606.
[215] Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med 1999;340:1888–99.
[216] Bresalier RS, Sandler RS, Quan H, et al. Adenomatous Polyp Prevention on Vioxx (APPROVe) Trial Investigators. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med 2005;352:1092–102.
[217] Solomon SD, McMurray JJ, Pfeffer MA, et al. Adenoma Prevention with Celecoxib (APC) Study Investigators. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. N Engl J Med 2005;352:1071–80.

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