



# COX-2 Signaling in the Tumor Microenvironment

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## Abstract

Tumorigenesis is a multistep, complicated process, and many studies have been completed over the last few decades to elucidate this process. Increasingly, many studies have shifted focus toward the critical role of the tumor microenvironment (TME), which consists of cellular players, cell–cell communications, and extracellular matrix (ECM). In the TME, cyclooxygenase-2 (COX-2) has been found to be a key molecule mediating the microenvironment changes. COX-2 is an inducible form of the enzyme that converts arachidonic acid into the signal transduction molecules (thromboxanes and prostaglandins). COX-2 is frequently expressed in many types of cancers and has been closely linked to its occurrence, progression, and prognosis. For example, COX-2 has been shown to (1) regulate tumor cell growth, (2) promote tissue invasion and metastasis, (3) inhibit apoptosis, (4) suppress antitumor immunity, and (5) promote sustainable angiogenesis. In this chapter, we summarize recent advances of studies that have evaluated COX-2 signaling in TME.

## Keywords

Cyclooxygenase-2 · Structure · Prostaglandin · Arachidonic acid · Tumor · Tumorigenesis · Microenvironment · Regulation · Cell growth · Invasion · Metastasis · Apoptosis · Immunity · Angiogenesis · NSAID

## 6.1 Introduction

Tumorigenesis is a multistep and complicated process, in which oncogenes and tumor-suppressor genes are going through successive mutations and eventually lead to enhanced proliferation and resistance to apoptosis. Currently, several major hallmarks of human tumor have been universally reported, including evading growth suppressors, gaining genome instability, promoting replicative immortality, resisting cell death, eliminating cell energy limitation, promoting metastasis, inducing angiogenesis, sustaining proliferative signals, evading immune destruction, and aggregating inflammation [1, 2].

During the past few decades, the understanding of tumorigenesis has greatly increased [3] and the focus of studies has shifted from the malignant cells themselves to the tumor microenvironment (TME) and the interactions between them. TME, which consists of extracellular

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matrix (ECM) and cellular players such as fibroblasts, endothelial cells, neuroendocrine cells, adipose cells, leukocytes and so on, and their interactions [4], helps tumors to acquire their invasive characters. In detail, the tumoral niche has increasingly been reported to dictate abnormal tissue functions and play an important role in the subsequent evolution of malignancies [5]. Scientists have also found that a healthy microenvironment could help maintain the healthy cellular status and protect against tumorigenesis and metastasis [3]. Many studies have shown tumors are not only a mass of proliferative malignant cells, but they also attract other stromal cells [6], vascular cells [7], and immune cells [8] by secreting cytokines, chemokines, and stimulatory growth factors. These factors released by tumor cells may recruit other cells to rebuild the new microenvironment. Such communication between tumor cells and their microenvironment may enhance metastatic capability and immortal proliferation, causing eventual death [1, 2].

One of the key factors in the TME that has been characterized is cyclooxygenase-2 (COX-2). COX proteins are membrane-bound proteins, located on the nuclear envelope, and luminal side of the endoplasmic reticulum is an important mediator of angiogenesis and inflammation. It has three isoforms: COX-1, COX-2, and COX-3 [9, 10]. COX-1, which is expressed in most tissues, is a housekeeping enzyme to maintain the basal level of prostaglandins (PGs) [11]. It also helps maintain the internal homeostasis by regulating the processes such as vascular smooth muscle functioning, cytoprotection of the gastric mucosa, platelet aggregation, and renal function [9]. COX-3 is reported as a variant of COX-1, and it is mainly present in the central nervous system [12, 13]. By contrast, COX-2 is an inducible form, usually undetected in normal tissues and cells [14] in which its basal expression only can be found in the central nervous system, kidney, stomach [15], and female reproductive organs [16]. By contrast, it is usually constantly expressed in many types of tumor tissues [14, 17], such as squamous cell carcinoma, adenocarcinoma, transitional cell carcinoma, cholangiocarcinoma, hepatocellular carcinoma, and endometrial carcinoma [18, 19].

As TME actively participates in the tumor metastasis and progression, and COX-2 is one of the critical inflammatory mediators deregulated in many tumors, therapeutic strategies targeting the COX-2 in TME may have great potential and be highly selective. Below, we will highlight the role of COX-2 signaling in the regulation of tumor progression in the TME and discuss its potential value in tumor therapy.

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## 6.2 Structure of COX-2

Human COX-2 is a homodimer of 581 amino acids, which encoded by COX-2 gene locates on the chromosome 1q25.2-q25.3 [20]. The dimerization of two 70 kDa subunits is necessary for catalytic activity and its own structural integrity [21]. Each subunit of COX-2 contains three domains to form the structure: a membrane-binding domain (residues 73–116), an N-terminal epidermal growth factor domain (residues 34–72), and a C-terminal catalytic domain which comprises the bulk of the protein [22–28]. The membrane-binding domain consists of four amphipathic  $\alpha$  helices, three of which lie in the same plane, whereas the last one extends into the catalytic domain [29]. These helices have aromatic and hydrophobic residues. Therefore, this structure could create a surface that interacts with the lipid bilayer [22].

The peroxidase active site lies at the top of an L-shaped channel on the opposite side of the membrane-binding domain. It contains the heme that positioned at the bottom of a shallow cleft. Other molecules could access the heme easily except the dome formed by hydrophobic amino acids covers part of the cleft. At the entrance of the channel is a lobby. It is a large space that narrows to a constriction. Inhibitors or substrates can only pass into the channel when the lobby is open. On top of the lobby, the channel is surrounded by hydrophobic residues [25, 26, 28]. The structure of the active site makes COX-2 only react with specific substrate but not a wide range of organic hydroperoxides [30]. Interestingly, although the preference of the peroxidase relies on hydrophobic dome, mutation of the dome residues affects little on substrate specificity or peroxidase activity [31].

## 6.3 The COX-2 Signaling

### 6.3.1 The COX-2/PGE Signaling

COX-2 is a rate-limiting [20] and short-living enzyme [16] that converts phospholipase A2 (PLA2)-mobilized arachidonic acid (AA) into the signal transduction molecules thromboxanes and prostaglandins (PGs) [32]. One principal product of COX-2 is prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), a mediator contributing to the modulation of several biological processes, including angiogenesis, immunity, pain, and tumorigenesis [33–35]. In the tumor formation process, COX-2 could be overexpressed in TME due to transcriptional or posttranscriptional malfunction [36, 37]. Thus, COX-2 is an important marker for tumor identification [14, 38]. Elevated expression of COX-2 and its major product PGE<sub>2</sub> has been reported to be inversely associated with patients' survival rate [39–41].

Recent advances in the role of COX-2 and PGEs in the pathogenesis of cancer have been described [9, 15, 42–44]. The main form of prostaglandin involved in many types of cancers is PGE<sub>2</sub>. PGE<sub>2</sub> can act on the receptors, for example, EP1, EP2, EP3, and EP4 to induce PGE<sub>2</sub> signal cascade, leading to changes of intracellular calcium, cAMP, and some inflammatory factors. As a result, physiological or pathological processes follow [45, 46]. Recent investigations support that PGE<sub>2</sub> may enhance progression of colorectal cancer [47–49], and EP4 is a therapeutic target for cancer therapy [50, 51]. COX-2-derived PGE<sub>2</sub> can also contribute to tumor development through several mechanisms including inhibition of apoptosis. However, the mechanisms by which PGE<sub>2</sub> regulates apoptosis are still largely unknown. The EP2 and EP4 receptors mediate their activities through cAMP production. Suppression of apoptosis by cAMP has been seen in intestinal cells through the induction of the IAP family member inhibitor of apoptosis 2 (IAP-2) [52, 53]. Therefore, further research is warranted to investigate the antiapoptotic effects of PGE<sub>2</sub> mediated through cAMP, which results in the induction of the IAP family member c-IAP2.

### 6.3.2 Cytokines and Other Compounds Regulating COX-2 Signaling

#### 6.3.2.1 IL-1 $\beta$ and TNF- $\alpha$

Cytokines and other compounds such as interleukin 1 $\beta$  (IL-1 $\beta$ ) and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) may promote expression of COX-2 mRNA and protein in human colorectal fibroblasts, profoundly in cancer-associated fibroblasts (CAFs) [54–56]. When stimulated with the pro-inflammatory cytokines IL-1 $\beta$  or TNF- $\alpha$ , orbital fibroblasts express high levels of COX-2 and PGE<sub>2</sub> [57]. Scientists have found that IL-1 $\beta$  or TNF- $\alpha$  promotes synthesis of PGE<sub>2</sub> by 25-fold in human colorectal fibroblasts (CCD-18Co) and five human colorectal fibroblast strains obtained at routine colonoscopies [58]. Greater levels of IL-1 $\beta$ -stimulated COX-2 expression and PGE<sub>2</sub> synthesis in the cancer-associated fibroblasts could only be accounted for partially by increased COX-2 promoter and transcriptional activity in the cancer-associated phenotype. We have noted that IL-1 $\beta$  and TNF- $\alpha$  induce mRNA overexpression of COX-2 and promote production of PGE<sub>2</sub> in human colorectal fibroblasts, especially in CRC-associated strains [54, 59] at a rate at which COX-2 mRNA decays can be dramatically retarded in vitro by PGE<sub>2</sub> [60].

#### 6.3.2.2 NF- $\kappa$ B

The nuclear factor (NF)- $\kappa$ B could also regulate the activation of COX-2 signaling in cancer cells [61]. The subfamily of NF- $\kappa$ B proteins has five members, including NF- $\kappa$ B1 (p50), NF- $\kappa$ B2 (p52), RelA (p65), RelB, and c-Rel [18, 62, 63]. Among the subfamily, p65 plays a role in the regulation of COX-2 in cancer cells [64, 65]. NF- $\kappa$ B/COX-2 signaling could be induced by protein kinase C (PKC) [66], TRIP4 [65], ERK1/2 [67], IL-1 $\beta$  [61], caspase-3 [68], and conditions like endoplasmic reticulum (ER) stress [69]. Inhibition of this signaling is mediated by annexin A5 [66] and miR-16 [70].

#### 6.3.2.3 PKC and MAPK

Cytokines and growth factors induce COX-2 expression via protein kinase C (PKC) signaling.

Molecules that interfere with microtubules such as taxanes could induce COX-2 by activating PKC and mitogen-activated protein kinases (MAPKs). There are three related MAPK proteins including ERK1/2, p38, and c-Jun N-terminal kinase, which are contributed to the induction of COX-2 [71]. These members could mediate PKC effects on COX-2 signaling in cancer cells [72]. Combination of PKC and COX-2 inhibitors can synergistically inhibit melanoma metastasis [73]. Among the MAPKs, p38 [74] and ERK1/2 [75] are downstream molecules of COX-2. In addition, COX-2/P38 signaling favors angiogenesis [74] and is involved in cancer cell resistance to apoptosis [76].

#### 6.3.2.4 Other Signaling

There are also many other cytokines and compounds which can regulate COX-2 signaling. One example is COX-2/STAT3 signaling, which contributes to the proliferation [77] and epithelial–mesenchymal transition (EMT) [78] of cancer cells by promoting the immunosuppressive microenvironment [75]. Another example is SDF-1a which plays a role in cancer cell metastasis and invasion through the stimulation of COX-2 by interaction with its receptor CXCR4 [79, 80]. All the research above suggests that COX-2 signaling is highly involved in the pathogenesis of cancer.

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## 6.4 COX-2 Signaling in Tumor Microenvironment (TME)

### 6.4.1 COX-2 Regulates the Tumor Cell Growth

The cell behavior is controlled by complex signaling pathways. It is thought that the malfunction of these signaling pathways causes tumor cells to grow uncontrollably. Two major signaling pathways, Ras-MAPK and the PI3K/AKT signaling, are frequently shown to be deregulated in many human cancers, which can stimulate cell growth and survival when activated [81, 82]. There is a strong evidence showing that COX-2, together with PGE<sub>2</sub>, are mediators of cancer cell growth through the above signaling [83]. PGE<sub>2</sub>

derived from COX-2 can enhance cell survival through the PI3K/AKT and Ras-MAPK/ERK signaling. Aberrant activation of the COX-2/PGE<sub>2</sub> signaling might increase mutations in the above two signaling pathways, which could promote tumor progression [84–86]. Furthermore, there are other ways mediating cancer cell growth by COX-2. For example, activation of stromal cancer-associated fibroblasts (CAFs) and neutrophils by COX-2 can release proliferative signals on cancer cells [87, 88], and induction of aromatase cytochrome P450 (CYP19) by COX-2 contributes to the conversion of estrogen to estrogen quinones [89], which is involved in tumor proliferation [90].

Under physiological conditions, normal tissue can control cell growth by the action of antiproliferative signals, which is a crucial mechanism for maintaining homeostasis [1]. The membrane-bound ligands and soluble growth inhibitors are two kinds of key compounds of above signals to repress cell growth. Scientists have demonstrated two antigrowth signals that can restrain proliferation and maintain tissue homeostasis [1]. However, deregulation of the COX-2/PGE<sub>2</sub> signaling may limit the function of these signals by additional mechanism. The first antigrowth signals can maintain cells in G0 state to block proliferation and keep cell quiescence. For example, transforming growth factor-beta (TGF-β) can block cell growth by activation of cyclin-dependent kinase inhibitors and suppression of c-Myc [91]. Usually, cancer cells are insensitive to the suppressive effect of TGF-β due to inactivated mutations of the receptors or downstream signaling effectors [91]. One study showed that mutations of TGF-β receptor type II occur in colorectal tumors at a high frequency [92]. However, these mutations do not exist in all types of cancer cells. It is also reported that overexpression of COX-2 can downregulate the expression of TGF-β receptor type II, which means COX-2 signaling can prevent the receipt of antigrowth signals [93]. The second antigrowth signals are to initiate a terminally differentiated state [1]. Aberrant activation of pathways such as β-catenin/WNT signaling in colorectal tumors contributes to the blockage of normal differentiation and maintenance of progenitor state of

cancer cells [94]. Recently, evidence demonstrates that the COX-2 signaling can activate the  $\beta$ -catenin/WNT signaling to keep cells in a progenitor state [95]. Furthermore, when there is lack of  $\beta$ -catenin/WNT mutations, inappropriate activation of the COX-2/PGE<sub>2</sub> signaling could discourage cell differentiation.

In addition to the above function, the activation of  $\beta$ -catenin/WNT signaling by PGE<sub>2</sub> might also serve to the acquisition of the immortal phenotype [95], which means it can help the cancer cells to get limitless replicative potential. For example, colorectal cancer is thought to start from such immortal cells initiated by mutations in the  $\beta$ -catenin/WNT signaling. Scientists demonstrated that in intestinal crypts, the stem cells and progenitor cells are maintained by activating WNT signaling [94]. Mutations of components of WNT signaling in colorectal tumors result in the formation of an active  $\beta$ -catenin/T-cell factor (TCF) complex that can mimic WNT signaling. It is reported that COX-2/PGE<sub>2</sub> signaling may play a role in keeping the crypt in the progenitor phenotype by activating  $\beta$ -catenin/TCF complex in colorectal cancer cells [95]. Perturbation of the WNT signaling by deleting TCF4 in mice also leads to loss of the stemness in the small intestine [96]. This suggests that the WNT signaling could maintain the crypt stem cell phenotype in both physiological and cancer status.

#### 6.4.2 COX-2 Promotes Tissue Invasion and Metastasis

COX-2 has been shown to be one of the critical metastasis progression genes [97] participating in the metastasis into the brain [98], bone [99], lymph nodes [100], and liver [101]. Factors like IL-11 induced by COX-2 are related to the cancer metastasis [99]. In order to achieve the invasion and metastasis, cancer cells must show an invasive phenotype of more motile status. They lose and detach themselves from connected cells within the tumor, move into extracellular matrix, and finally invade into blood vessels and lymphatics [102]. After escaping from the primary tumor tissue, cancer cells must then colonize the surrounding tissue or distant sites with the help of

blood or lymphatics. Recently, the significance of COX-2 as a necessary mediator for dissemination of cancer cells was reported in an *in vivo* model of breast cancer metastasis to the lungs [103]. Using both pharmacological and genetic methods, this study demonstrated that COX-2 is one of the key “metastasis” genes which helps to mediate tumor development, invasion, and metastasis to other tissues.

There are many other studies demonstrating that COX-2 signaling plays critical roles in the metastasis processes—more specifically, promoting a more metastatic phenotype in colorectal tumor cells through its product PGE<sub>2</sub>. For example, EGFR transactivation mediated by intracellular Src can stimulate the motility and invasion controlled by PGE<sub>2</sub> [104]. PGE<sub>2</sub> could also promote cytoskeletal reorganization and eventually lead to invasion and migration of colorectal cancer cells via PI3K signaling [105]. Overexpression of COX-2 can modulate the adhesive properties of intestinal cells [93] and increase the activity of matrix metalloproteinase (MMP) to promote tumor invasion [106]. Inhibition of this marker can prevent the metastasis of colorectal tumors *in vivo* in both human [107] and mice [108]. In addition, c-Met, also known as the hepatocyte growth factor receptor, is transactivated by PGE<sub>2</sub> through an EGFR-dependent pathway in colorectal cancer [109]. C-Met signaling is associated with the loss of cell contact and invasive growth [110]. Scientists found that COX-2, c-Met, and  $\beta$ -catenin coexist at the invasive edge of colorectal tumor [109]. The transactivation of c-Met can induce nuclear accumulation of  $\beta$ -catenin and increase expression and invasion of urokinase-type plasminogen activator receptor through Matrigel [109]. COX-2 can also induce  $\beta$ 1-integrin that is related to cancer cell invasion [111, 112].

Furthermore, COX-2 can induce epithelial-mesenchymal transition (EMT) through factors like transcription-3 (STAT3) and miR526b [78, 113]. In cancer cells, EMT is thought to be a promoter of invasiveness [18]. Inhibition of EMT mediated by COX-2 occurs after usage of cannabinoids in cancer [114]. Interestingly, in the TME, the tumor maintenance and progression are only regulated by COX-2 secreted by the tumor



cells but not by other normal cells such as stromal cells [115, 116]. Therefore, these findings suggest that COX-2 plays an important role in tumorigenesis.

### 6.4.3 COX-2 Inhibits Apoptosis

Apoptosis, the cell death programming process [117], plays an essential role in controlling cell number and maintaining tissue homeostasis in normal tissue [118, 119]. Malfunction of this mechanism results in excessive cell number and survival rate, which can lead to tumorigenesis and its malignant progression [120–122]. COX-2 is related to suppression of apoptosis in many cancer types. The ability of COX-2/PGE<sub>2</sub> signaling to control apoptosis in tumor cells may depend on factors such as the TME and vary between cell types. In this signaling, several mechanisms have been reported. COX-2 contributes to the cancer apoptosis resistance through delaying G1 phase to slow the cell cycle [123]. It also induces the expressions of BCL-2 [124, 125], MCL-1 [126], and Survivin [127] and represses caspase-3 signaling [128].

First, overexpression of COX-2 might regulate the intrinsic apoptosis signaling by inducing the expression of BCL-2 and increase resistance apoptosis induced by butyrate in rat intestinal epithelial cells [93]. Later studies demonstrated that COX-2/PGE<sub>2</sub> might suppress apoptosis by increasing the expression of BCL-2 through activation of Ras-MAPK/ ERK signaling [129]. Other studies also indicated that COX-2 signaling controls apoptosis by inducing the expressions of BCL-2 [124, 125]. Second, scientists found that COX-2 is a critical mediator in apoptosis resistance by increasing the expression of MCL-1 [126]. Knockdown of MCL-1 would sensitize the lung cancer cells to apoptosis substantially. Moreover, the expression of MCL-1 could be significantly decreased when COX-2 was suppressed [126]. Third, it was reported that overexpression of COX-2 contributes to the expression and stabilization of Survivin, which is an inhibitor of apoptosis in non-small-cell lung cancer [127]. Suppression of COX-2 activity could induce degradation of Survivin and lead to lower

cellular response to apoptosis pathways [127]. Fourth, scientists have reported that overexpression of COX-2 limited the cleavage of HuR and caspase-3, which reduced cell apoptosis in the paclitaxel-resistant oral cancer cells [128]. They also showed that inhibition of COX-2 increased apoptosis in paclitaxel-resistant oral cancer cells by activating of caspase-3, both in vivo and in vitro [128]. Furthermore, studies also demonstrate that COX-2/PGE<sub>2</sub> signaling might regulate apoptotic by involving in many other pathways. For example, it is reported that PGE<sub>2</sub> activates prosurvival signaling, such as ERK signaling [130], PI3K/AKT signaling [105, 131], EGFR signaling [132, 133], and cAMP/PKA signaling [134].

Other conditions like hypoxia could also contribute to the induction of cell death. For example, in colorectal tumor cells, COX-2/PGE<sub>2</sub> signaling could promote cell survival in hypoxia condition by activation of Ras-MAPK signaling [86], suggesting that COX-2 plays an important role in promoting the survival rate of cancer cells under difficult microenvironmental conditions. In addition, wild-type p53 is a suppressor of COX-2 in mediating apoptosis [18, 36]. Mutations of p53 in cancer cells would create a positive-feedback loop between COX-2 and itself. It might be a chemotherapeutic target for cancers [36, 135].

### 6.4.4 COX-2 Suppresses Antitumor Immunity

COX-2 signaling plays an important role in immune resistance and cancer immunotherapy. It regulates the immune response through recruiting immune cells into the tumor milieu to induce an immunosuppressive state [136]. Cancer cells can release COX-2/PGE<sub>2</sub> to the milieu to suppress immunological responses by blocking the activity of cytotoxic T lymphocytes [137]. COX-2/PGE<sub>2</sub> has also been shown to be a major modulator of macrophage activation for a long time [138]. One of the major populations of tumor-infiltrating immune cells is tumor-associated macrophages (TAMs). Reprogramming the TAMs of M2 toward M1

phenotype or impeding the process toward the pro-tumor M2 subtype is an anticancer strategy [44]. COX-2/PGE<sub>2</sub> signaling could promote macrophage differentiating to M2 subtype [139, 140]. Immune suppression regulated by macrophages is related to increased T-cell infiltration regulated by CD4+/CD25+ and decreased CD8+ T-cell function [44].

Overexpression of COX-2 promotes tumorigenesis by inhibiting proliferation of B-type and T-type lymphocytes, especially natural killer T cells, and subsequently limits immunosuppression of the host [141]. COX-2 inhibits the exposure of antigen-specific T cells to their cellular targets and promotes the expression of indoleamine 2,3-dioxygenase and interleukin-4 (IL-4) by tumor cells [44]. Scientists have demonstrated that COX-2/PGE<sub>2</sub> is the factor resisted to the cytotoxicity induced by active form of antigen-specific T cells [142]. It has also been shown that T-cell receptors (TCR) such as TCR NKG2D (natural-killer group 2, member D), V $\gamma$ 9V $\delta$ 2 (V $\delta$ 2 gene with the co-expression of the V $\gamma$ 9 chain), and CD16 are all inhibited by COX-2/PGE<sub>2</sub> [143]. Moreover, COX-2/PGE<sub>2</sub> helps the immune suppression mediated by cancer. They play an important role in promoting CD4+ and CD8+ T-cell differentiation and directly inhibiting the proliferation and effector functions of regulatory T cells [144]. Furthermore, it is reported that Treg cells inhibited effector T cells by activating COX-2 signaling and participated in cancer immunosuppression [145, 146]. The expression of COX-2 is also significantly related to Treg localization and prevalence [147]. In addition, expression of the forkhead/winged helix transcription factor (FOXP3) gene could also drive the suppressive activity of regulatory T cells.

Natural killer (NK) cells are a subpopulation of lymphocytes that take part in innate immunity. All types of PGE<sub>2</sub> receptors are expressed by NK cells, and PGE<sub>2</sub> derived from tumor is a critical barrier to the NK cell-mediated killing. It has been reported that the natural cytotoxicity receptors (NCRs), such as NKP30, NKP44, NKP46, major NK receptors (NKR), NKG2D, and CD16, could all be inhibited by PGE<sub>2</sub> [143]. In addition, the function of NK cells such as secrete

interferon- $\gamma$  (INF- $\gamma$ ), exert cytotoxic effects, and migrate are all inhibited by PGE<sub>2</sub> [148]. EP2 and EP4 are the major receptors acted by PGE<sub>2</sub> while inhibiting NK cells. And frondoside A, an EP4 antagonist, inhibits breast tumor metastasis by acting on NK cells and decreases INF- $\gamma$  production by NK cells [44]. Furthermore, MDSC presents in many cancer types and blocks adaptive immunity by inhibiting NK cells and the activation of CD4+ and CD8+ T cells [148, 149]. COX2 produced by tumor cells would maintain high level of MDSC, and subsequently block the tumor immunity. It has been shown to allow the proliferation of tumor cells without control from the immune system of the host [44].

Dendritic cells (DCs) participate in both innate and adaptive immunity. COX-2 is a crucial immunomodulator of DC activities [150], which can reduce DC ability to present antigens, express MHC class II molecules, mature, and activate T cells [151]. COX-2/PGE<sub>2</sub> has been demonstrated to decrease the cytokine production of antigen-presenting DCs, away from a type 1 T cell (Th1) profile, and eventually result in a reduced antitumor activation of cytotoxic CD8+ T cells [152, 153]. Meanwhile, it is reported that EP2 and EP4 receptor subtypes of PGE<sub>2</sub> may be targets of modulating DC activity [90]. For example, PGE<sub>2</sub> could increase interleukin-10 (IL-10) production, which can lead to downregulation of DC functions. These abilities of COX-2/PGE<sub>2</sub> signaling to suppress antitumor immune responses may allow malignant cells to escape immunosurveillance and promote tumor development.

#### 6.4.5 COX-2 Promotes Sustainable Angiogenesis

COX-2 induced in tumor is associated with angiogenesis [154]. Inhibition of COX-2 suppresses corneal neovascularization in experimental lung and colon tumor growth [155]. COX-2 expression localizes in tumor epithelium [106], stromal fibroblasts [115], endothelium [155], and infiltrating immune cells [156]. It also promotes the production of vascular endothelial growth factor (VEGF), a potent angiogenic growth factor [157]. It was demonstrated that expression of

COX-2 was critical for the induction of VEGF and the subsequent tumor angiogenesis in an *Apc*/COX-2 double-knockout mice model [158]. It is also reported that in COX-2 knockout mice, fibroblasts showed decreased level of VEGF mRNA and protein, together with lower vascular density compared to wild-type mice [115]. Consistent with this, *in vivo* studies have showed that homozygous deletion of COX-2 led to slower growth of tumor xenografts and lower tumor vascular density [115]. One possible mechanism is that COX-2 might promote tumor angiogenesis through the production of PGE<sub>2</sub>, which has been reported to involve in endothelial cell spreading and migration by activation of Cdc42 and Rac [159]. PGE<sub>2</sub> has also been demonstrated to induce VEGF expression in colon cancer cells by activating HIF-1, one of the key regulators of VEGF expression [160]. Furthermore, PGE<sub>2</sub> has been reported to regulate vascularization through chemokine receptor signaling. For example, *in vivo* model showed that PGE<sub>2</sub> can enhance basic fibroblast growth factor-induced chemokine receptor-4 that is crucial for vessel assembly [161]. Moreover, PGE<sub>2</sub> can stimulate the expression of CXCL-1 *in vivo*, a pro-angiogenic chemokine [162].

In addition, COX-2 modifies molecules involved in endothelial trafficking with vascular mural cells/pericytes, an interaction critical to vessel stability [163–165]. Pericytes are found in all vascularized tissues, attaching to the walls of blood vessels [166]. They surround vascular endothelial cells and communicate with them by physical contacts and paracrine signaling along the length of the blood vessels [167, 168]. Increased expression of key modulator of pericyte PDGF- $\beta$  or enhanced pericytes recruitment is characteristic features of tumor vasculature [169–171]. Moreover, when transplanting cancer cells into Nestin-GFP/NG2-DsRed mice, type-2 pericytes were recruited during the angiogenesis of the development of tumor, while type-1 pericytes did not penetrate [172]. COX-2, which modifies the proliferation and function of pericytes, plays a crucial role in vascular response to chronic microenvironmental stress [173, 174]. A study in 2006 demonstrated the function of COX-2 in vascular assembly in an orthotopic

xenograft model by using the specific COX-2 inhibitor SC-236. The results showed that tumor growth was suppressed by SC-236 significantly in human Wilms' tumor [164]. All the evidence above suggests that COX-2 could promote sustainable angiogenesis in tumor.

#### 6.4.6 Regulation of COX-2 Expression by the TME

Upregulation of COX-2 has been described in many different types of tumors [175]. It is reported that the TME is a promoter of COX-2 overexpression [36]. This overexpression is led by uncontrolled function of transcriptional or posttranscriptional levels [37]; therefore, it could be an important marker to identify tumor cells from normal tissues [14, 38]. Although PTGS2 (the gene-encoding human COX-2) mutations have not been described clearly, there are several known mechanisms which can promote expression of COX-2 in tumor cells. In general, the mechanisms can be divided into two types: oncogene activation and growth factor signaling deregulation. For example, it is reported that the hypoxic microenvironment can induce COX-2 expression in colorectal tumor cells [86]. This upregulation is mediated by HIF-1, a regulator of transcription in hypoxia. The same regulation dependent on HIF-1 has also been reported in lung cancer cells [176]. Other examples include activation of the TGF- $\beta$  receptors [177], gastrin receptors [178], c-Met [179],  $\beta$ -catenin/WNT signaling [180, 181], and the Ras-MAPK pathway [85, 182]. In addition, COX-2 is a constituent of exosomes derived from tumor [183]. Cancer promoters [184], oncogenic viruses [61], proinflammatory cytokines [185], radiation [186], and chemotherapy [187] are all inducers of COX-2 expression in cancer cells.

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### 6.5 Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

For decades, significant progress has been achieved in the discovery of effective drugs for colorectal cancer. One of those is nonsteroidal



anti-inflammatory drugs (NSAIDs) which inhibit COX-2 [188, 189]. Examples of NSAID include aspirin, ibuprofen, naproxen, nimesulide, and sulindac acid. Different NSAIDs may act via different signaling pathways to interact with COX-2. For example, ibuprofen, indomethacin, and naproxen can bind the activity site of COX-2 and inhibit its activity reversibly, while aspirin acetylates the activity site of COX-2, attenuating its activity irreversibly. Some NSAIDs, for example, aspirin, can facilitate the effect of COX-2 inhibitors for treatment of stage III colorectal cancer [190]. In fact, aspirin may reduce colon cancer mortality in women by as much as 50% [191–193]. Recently, a hybrid drug KSS19, a combination of NSAID rofecoxib and cis-stilbene, has been found to be a potent COX-2 inhibitor, which inhibits colon cancer cell growth effectively [194].

Although COX-2 inhibitors are promising candidates for treatment of cancer, some concerns for treatment of cancer by COX inhibitors have been raised. For example, an elevated risk of myocardial infarction may be linked to its usage [195]. In addition, the extended use of nonselective NSAIDs is also associated with certain pathological symptoms, for example, abdominal pain, dyspepsia, gastritis, gastrointestinal bleeding, nausea, and perforation of gastroduodenal ulcers [196]. Therefore, no major clinical trials of those inhibitors were successfully completed due to concerns of their adverse effects. Nonetheless, NSAIDs are effective in certain degrees for prevention and treatment of cancer. For example, a randomized trial demonstrated that NSAIDs are preventive for colorectal cancer with polyps [197, 198]. According to the results of large-scale trials, including the Adenomatous Polyp Prevention on Vioxx trial [199], the Adenoma Prevention with Celecoxib trial [198], the Prevention of Colorectal Sporadic Adenomatous Polyps trial [200], and colon polyp prevention trial [201], COX-2 inhibitors are effective for prevention of recurrence from sporadic colon cancer. Regular consumption of NSAIDs is also helpful for low-

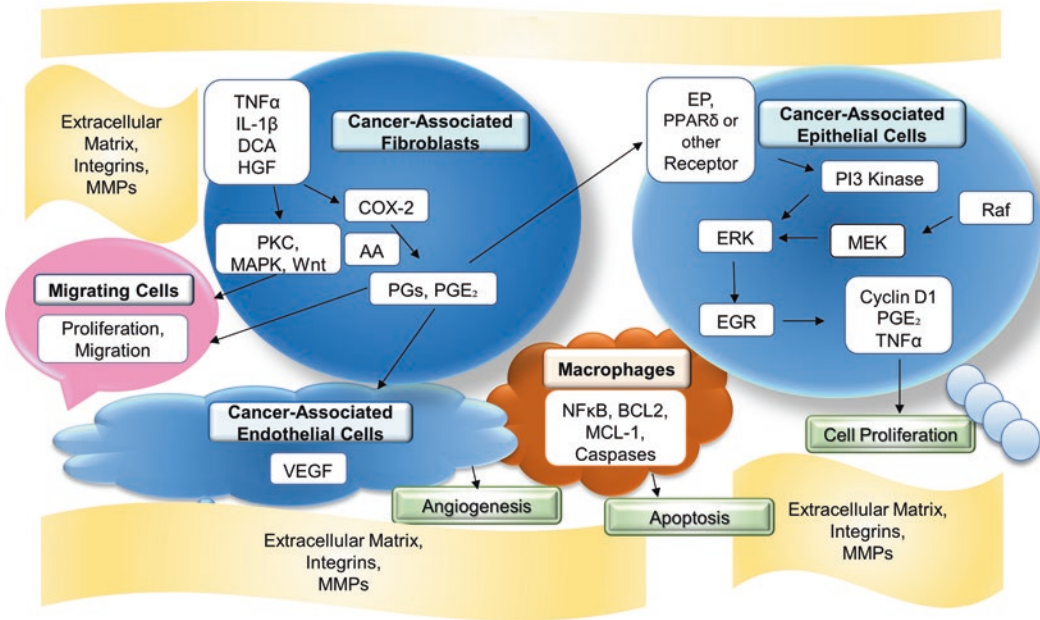
ering the risk of colorectal, breast, lung, and prostate cancer [202]. In all, COX inhibitors have shown promise, but there are still safety concerns.

To decrease the risk from COX inhibitors, many researchers have used low dose of COX inhibitors with other NSAIDs that target other critical pathways in carcinogenesis. For example, combination of celecoxib with erlotinib (an EGFR tyrosine kinase inhibitor) is more effective to control polyp formation using an ApcMin/+ mice model and to inhibit cancer growth in a xenograft model [203]. Celecoxib with erlotinib treatment is more effective for treatment of the advanced non-small-cell lung cancer [204]. A 5-lipoxygenase inhibitor has been shown to inhibit resistant tumor cells to SC-236 (COX inhibitor) and tumor growth in a breast cancer animal model [205]. Combined treatment of celecoxib with peroxisome proliferators-activated receptor- $\gamma$  agonist has been shown better than either alone in a mouse breast cancer model [206]. Combination of aromatase inhibitors with celecoxib has been shown better for patients suffering from metastatic breast cancer than either alone [207]. Therefore, we may like to reconsider the prospect of COX inhibitors for treatment of cancer.

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## 6.6 Conclusion and Perspective

As studies have shown over the last few decades, COX-2 is one of the key markers indicating worse cancer prognosis and stimulates cancer via various roles in the TME. To date, clinical and basic research has shown that reduction of PGE<sub>2</sub> synthesis by either specific COX-2 inhibitors or NSAIDs has the potential to decrease the risk of tumorigenesis of certain types [97, 208–214]. Therefore, therapeutic strategies targeting the COX-2 in the TME may have great potential to improve clinical outcomes. COX-2 signaling in the tumor environment is summarized as follows (Fig. 6.1):



**Fig. 6.1** COX-2 signaling in the tumor environment

## References

- Hanahan D, Weinberg RA (2000) The hallmarks of cancer. *Cell* 100(1):57–70
- Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144(5):646–674
- Wang M, Zhao J, Zhang L, Wei F, Lian Y, Wu Y et al (2017) Role of tumor microenvironment in tumorigenesis. *J Cancer* 8(5):761–773
- Chen F, Zhuang X, Lin L, Yu P, Wang Y, Shi Y et al (2015) New horizons in tumor microenvironment biology: challenges and opportunities. *BMC Med* 13:45
- Mroue R, Bissell MJ (2013) Three-dimensional cultures of mouse mammary epithelial cells. *Methods Mol Biol* 945:221–250
- Cirri P, Chiarugi P (2011) Cancer associated fibroblasts: the dark side of the coin. *Am J Cancer Res* 1(4):482–497
- Cheng L, Huang Z, Zhou W, Wu Q, Donnola S, Liu JK et al (2013) Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. *Cell* 153(1):139–152
- Gabrilovich DI, Ostrand-Rosenberg S, Bronte V (2012) Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* 12(4):253–268
- Pang LY, Hurst EA, Argyle DJ (2016) Cyclooxygenase-2: a role in cancer stem cell survival and repopulation of cancer cells during therapy. *Stem Cells Int* 2016:2048731
- Claria J (2003) Cyclooxygenase-2 biology. *Curr Pharm Des* 9(27):2177–2190
- Soh JW, Weinstein IB (2003) Role of COX-independent targets of NSAIDs and related compounds in cancer prevention and treatment. *Prog Exp Tumor Res* 37:261–285
- Sarkar FH, Adsule S, Li Y, Padhye S (2007) Back to the future: COX-2 inhibitors for chemoprevention and cancer therapy. *Mini Rev Med Chem* 7(6):599–608
- Kis B, Snipes JA, Isse T, Nagy K, Busija DW (2003) Putative cyclooxygenase-3 expression in rat brain cells. *J Cereb Blood Flow Metab* 23(11):1287–1292
- Gurram B, Zhang S, Li M, Li H, Xie Y, Cui H et al (2018) Celecoxib conjugated fluorescent probe for identification and discrimination of Cyclooxygenase-2 enzyme in cancer cells. *Anal Chem* 90(8):5187–5193
- Su CW, Zhang Y, Zhu YT (2016) Stromal COX-2 signaling are correlated with colorectal cancer: a review. *Crit Rev Oncol Hematol* 107:33–38
- Obermoser V, Baecker D, Schuster C, Braun V, Kircher B, Gust R (2018) Chlorinated cobalt alkyne complexes derived from acetylsalicylic acid as new specific antitumor agents. *Dalton Trans* 47(12):4341–4351
- Raj V, Bhaduria AS, Singh AK, Kumar U, Rai A, Keshari AK et al (2018) Novel 1,3,4-thiadiazoles inhibit colorectal cancer via blockade of IL-6/COX-2 mediated JAK2/STAT3 signals as evidenced through data-based mathematical modeling. *Cytokine* 118:144
- Mortezaei K (2018) Human hepatocellular carcinoma: protection by melatonin. *J Cell Physiol* 233(10):6486–6508

19. Dannenberg AJ, Lippman SM, Mann JR, Subbaramaiah K, DuBois RN (2005) Cyclooxygenase-2 and epidermal growth factor receptor: pharmacologic targets for chemoprevention. *J Clin Oncol* 23(2):254–266
20. Xu W, Huang Y, Zhang T, Zhao L, Fan J, Li L (2018) Cyclooxygenase-2 gene polymorphisms and susceptibility to hepatocellular carcinoma: a meta-analysis based on 10 case-control studies. *J Cancer Res Ther* 14(Supplement):S105–SS13
21. Xiao G, Chen W, Kulmacz RJ (1998) Comparison of structural stabilities of prostaglandin H synthase-1 and -2. *J Biol Chem* 273(12):6801–6811
22. Picot D, Loll PJ, Garavito RM (1994) The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. *Nature* 367(6460):243–249
23. Luong C, Miller A, Barnett J, Chow J, Ramesha C, Browner MF (1996) Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. *Nat Struct Biol* 3(11):927–933
24. Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY et al (1996) Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature* 384(6610):644–648
25. Smith WL, DeWitt DL, Garavito RM (2000) Cyclooxygenases: structural, cellular, and molecular biology. *AnnuRevBiochem* 69:145–182
26. Rouzer CA, Marnett LJ (2003) Mechanism of free radical oxygenation of polyunsaturated fatty acids by cyclooxygenases. *Chem Rev* 103(6):2239–2304
27. Mbonye UR, Yuan C, Harris CE, Sidhu RS, Song I, Arakawa T et al (2008) Two distinct pathways for cyclooxygenase-2 protein degradation. *J Biol Chem* 283(13):8611–8623
28. Garavito RM, Malkowski MG, DeWitt DL (2002) The structures of prostaglandin endoperoxide H synthases-1 and -2. *Prostaglandins Other Lipid Mediat* 68-69:129–152
29. Garavito RM, Mulichak AM (2003) The structure of mammalian cyclooxygenases. *AnnuRevBiophysBiomolStruct* 32:183–206
30. Kulmacz RJ, van der Donk WA, Tsai AL (2003) Comparison of the properties of prostaglandin H synthase-1 and -2. *Prog Lipid Res* 42(5):377–404
31. Liu J, Seibold SA, Rieke CJ, Song I, Cukier RI, Smith WL (2007) Prostaglandin endoperoxide H synthases: peroxidase hydroperoxide specificity and cyclooxygenase activation. *J Biol Chem* 282(25):18233–18244
32. van der Donk WA, Tsai AL, Kulmacz RJ (2002) The cyclooxygenase reaction mechanism. *Biochemistry* 41(52):15451–15458
33. Smith WL, DeWitt DL, Garavito RM (2000) Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem* 69:145–182
34. Howe LR (2007) Inflammation and breast cancer. Cyclooxygenase/prostaglandin signaling and breast cancer. *Breast Cancer Res* 9(4):210
35. Singh-Ranger G, Salhab M, Mokbel K (2008) The role of cyclooxygenase-2 in breast cancer: review. *Breast Cancer Res Treat* 109(2):189–198
36. Ohtsuka J, Oshima H, Ezawa I, Abe R, Oshima M, Ohki R (2018) Functional loss of p53 cooperates with the in vivo microenvironment to promote malignant progression of gastric cancers. *Sci Rep* 8(1):2291
37. Liu Y, Borchert GL, Surazynski A, Phang JM (2008) Proline oxidase, a p53-induced gene, targets COX-2/PGE2 signaling to induce apoptosis and inhibit tumor growth in colorectal cancers. *Oncogene* 27(53):6729–6737
38. Yue X, Nguyen TD, Zellmer V, Zhang S, Zorlutuna P (2018) Stromal cell-laden 3D hydrogel microwell arrays as tumor microenvironment model for studying stiffness dependent stromal cell-cancer interactions. *Biomaterials* 170:37–48
39. Gallo O, Masini E, Bianchi B, Bruschini L, Paglierani M, Franchi A (2002) Prognostic significance of cyclooxygenase-2 pathway and angiogenesis in head and neck squamous cell carcinoma. *Hum Pathol* 33(7):708–714
40. Jiao G, Ren T, Lu Q, Sun Y, Lou Z, Peng X et al (2013) Prognostic significance of cyclooxygenase-2 in osteosarcoma: a meta-analysis. *Tumour Biol: the journal of the International Society for Oncodevelopmental Biology and Medicine* 34(5):2489–2495
41. Sicking I, Rommens K, Battista MJ, Bohm D, Gebhard S, Lebrecht A et al (2014) Prognostic influence of cyclooxygenase-2 protein and mRNA expression in node-negative breast cancer patients. *BMC Cancer* 14:952
42. Roelofs HM, Te Morsche RH, van Heumen BW, Nagengast FM, Peters WH (2014) Over-expression of COX-2 mRNA in colorectal cancer. *BMC Gastroenterol* 14:1
43. Liu Y, Sun H, Hu M, Zhang Y, Chen S, Tighe S et al (2017) The role of Cyclooxygenase-2 in colorectal carcinogenesis. *Clin Colorectal Cancer* 16(3):165–172
44. Liu B, Qu L, Yan S (2015) Cyclooxygenase-2 promotes tumor growth and suppresses tumor immunity. *Cancer Cell Int* 15:106
45. Zhu Y, Hua P, Lance P (2003) Cyclooxygenase-2 expression and prostanoid biogenesis reflect clinical phenotype in human colorectal fibroblast strains. *Cancer Res* 63(2):522–526
46. Prescott SM, Fitzpatrick FA (2000) Cyclooxygenase-2 and carcinogenesis. *BiochimBiophysActa* 1470(2):M69–M78
47. Fujino H (2016) The roles of EP4 prostanoid receptors in cancer malignancy signaling. *Biol Pharm Bull* 39(2):149–155
48. Fujino H, Seira N, Kurata N, Araki Y, Nakamura H, Regan JW et al (2015) Prostaglandin E2-stimulated prostanoid EP4 receptors induce prolonged de novo prostaglandin E2 synthesis through biphasic phosphorylation of extracellular signal-regulated kinases mediated by activation of protein kinase A in HCA-7 human colon cancer cells. *Eur J Pharmacol* 768:149–159

49. Thomas SS, Makar KW, Li L, Zheng Y, Yang P, Levy L et al (2015) Tissue-specific patterns of gene expression in the epithelium and stroma of normal colon in healthy individuals in an aspirin intervention trial. *Genom Data* 6:154–158
50. Lin MC, Chen SY, He PL, Herschman H, Li HJ (2018) PGE2 /EP4 antagonism enhances tumor chemosensitivity by inducing extracellular vesicle-mediated clearance of cancer stem cells. *Int J Cancer* 143:1440
51. Lala PK, Nandi P, Majumder M (2018) Roles of prostaglandins in tumor-associated lymphangiogenesis with special reference to breast cancer. *Cancer Metastasis Rev* 37:369
52. Crowley-Weber CL, Payne CM, Gleason-Guzman M, Watts GS, Futscher B, Waltmire CN et al (2002) Development and molecular characterization of HCT-116 cell lines resistant to the tumor promoter and multiple stress-inducer, deoxycholate. *Carcinogenesis* 23(12):2063–2080
53. Nishihara H, Kizaka-Kondoh S, Insel PA, Eckmann L (2003) 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 U S A* 100(15):8921–8926
54. Zhu Y, Zhu M, Lance P (2012) IL1beta-mediated Stromal COX-2 signaling mediates proliferation and invasiveness of colonic epithelial cancer cells. *Exp Cell Res* 318(19):2520–2530
55. Zhu Y, Zhu M, Lance P (2012) iNOS signaling interacts with COX-2 pathway in colonic fibroblasts. *Exp Cell Res* 318(16):2116–2127
56. Zhu Y, Zhu M, Lance P (2012) Stromal COX-2 signaling activated by deoxycholic acid mediates proliferation and invasiveness of colorectal epithelial cancer cells. *Biochem Biophys Res Commun* 425(3):607–612
57. Wang HS, Cao HJ, Winn VD, Rezanka LJ, Frobert Y, Evans CH et al (1996) Leukoregulin induction of prostaglandin-endoperoxide H synthase-2 in human orbital fibroblasts. An in vitro model for connective tissue inflammation. *J Biol Chem* 271(37):22718–22728
58. Kim EC, Zhu Y, Andersen V, Sciaky D, Cao HJ, Meekins H et al (1998) Cytokine-mediated PGE2 expression in human colonic fibroblasts. *Am J Physiol* 275(4 Pt 1):C988–C994
59. Zhu M, Zhu Y, Lance P (2013) TNFalpha-activated stromal COX-2 signalling promotes proliferative and invasive potential of colon cancer epithelial cells. *Cell Prolif* 46(4):374–381
60. Dixon DA, Tolley ND, King PH, Nabors LB, McIntyre TM, Zimmerman GA et al (2001) Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. *J Clin Invest* 108(11):1657–1665
61. Charalambous GP, Maihofner C, Bhambra U, Lightfoot T, Goderham NJ (2003) Colorectal Cancer Study G. Upregulation of cyclooxygenase-2 is accompanied by increased expression of nuclear factor-kappa B and I kappa B kinase-alpha in human colorectal cancer epithelial cells. *Br J Cancer* 88(10):1598–1604
62. Mortezaee K, Khanlarkhani N (2018) Melatonin application in targeting oxidative-induced liver injuries: A review. *J Cell Physiol* 233(5):4015–4032
63. Mortezaee K, Khanlarkhani N, Beyer C, Zendedel A (2018) Inflammasome: its role in traumatic brain and spinal cord injury. *J Cell Physiol* 233(7):5160–5169
64. Cai TT, Ye SB, Liu YN, He J, Chen QY, Mai HQ et al (2017) LMP1-mediated glycolysis induces myeloid-derived suppressor cell expansion in nasopharyngeal carcinoma. *PLoS Pathog* 13(7):e1006503
65. Hao J, Xu H, Luo M, Yu W, Chen M, Liao Y et al (2018) The tumor-promoting role of TRIP4 in melanoma progression and its involvement in response to BRAF-targeted therapy. *J Invest Dermatol* 138(1):159–170
66. Baek HS, Park N, Kwon YJ, Ye DJ, Shin S, Chun YJ (2017) Annexin A5 suppresses cyclooxygenase-2 expression by downregulating the protein kinase C-zeta-nuclear factor-kappaB signaling pathway in prostate cancer cells. *Oncotarget* 8(43):74263–74275
67. Wong JH, Ho KH, Nam S, Hsu WL, Lin CH, Chang CM et al (2017) Store-operated Ca(2+) entry facilitates the lipopolysaccharide-induced cyclooxygenase-2 expression in gastric cancer cells. *Sci Rep* 7(1):12813
68. Feng X, Yu Y, He S, Cheng J, Gong Y, Zhang Z et al (2017) Dying glioma cells establish a proangiogenic microenvironment through a caspase 3 dependent mechanism. *Cancer Lett* 385:12–20
69. Hung JH, Su JJ, Lei HY, Wang HC, Lin WC, Chang WT et al (2004) Endoplasmic reticulum stress stimulates the expression of cyclooxygenase-2 through activation of NF-kappaB and pp38 mitogen-activated protein kinase. *J Biol Chem* 279(45):46384–46392
70. Liu X, Li S, Li Y, Cheng B, Tan B, Wang G (2018) Puerarin inhibits proliferation and induces apoptosis by upregulation of miR-16 in bladder cancer cell line T24. *Oncol Res* 26(8):1227–1234
71. Liu W, Reinmuth N, Stoeltzing O, Parikh AA, Tellez C, Williams S et al (2003) Cyclooxygenase-2 is up-regulated by interleukin-1 beta in human colorectal cancer cells via multiple signaling pathways. *Cancer Res* 63(13):3632–3636
72. Chou WY, Chuang KH, Sun D, Lee YH, Kao PH, Lin YY et al (2015) Inhibition of PKC-induced COX-2 and IL-8 expression in human breast cancer cells by glucosamine. *J Cell Physiol* 230(9):2240–2251
73. Zhou P, Qin J, Li Y, Li G, Wang Y, Zhang N et al (2017) Combination therapy of PKCzeta and COX-2 inhibitors synergistically suppress melanoma metastasis. *J Exp Clin Cancer Res* 36(1):115
74. Hu H, Han T, Zhuo M, Wu LL, Yuan C, Wu L et al (2017) Elevated COX-2 expression promotes angiogenesis through EGFR/p38-MAPK/Sp1-dependent signalling in pancreatic cancer. *Sci Rep* 7(1):470



75. Maturu P, Jones D, Ruteshouser EC, Hu Q, Reynolds JM, Hicks J et al (2017) Role of cyclooxygenase-2 pathway in creating an immunosuppressive micro-environment and in initiation and progression of Wilms' tumor. *Neoplasia* 19(3):237–249
76. Semaan J, Pinon A, Rioux B, Hassan L, Limami Y, Pouget C et al (2016) Resistance to 3-HTMC-induced apoptosis through activation of PI3K/Akt, MEK/ERK, and p38/COX-2/PGE2 pathways in human HT-29 and HCT116 colorectal cancer cells. *J Cell Biochem* 117(12):2875–2885
77. Ramu A, Kathiresan S, Ramadoss H, Nallu A, Kaliyan R, Azamuthu T (2018) Gramine attenuates EGFR-mediated inflammation and cell proliferation in oral carcinogenesis via regulation of NF-kappaB and STAT3 signaling. *Biomed Pharmacother* 98:523–530
78. Tong D, Liu Q, Liu G, Xu J, Lan W, Jiang Y et al (2017) Metformin inhibits castration-induced EMT in prostate cancer by repressing COX2/PGE2/STAT3 axis. *Cancer Lett* 389:23–32
79. Zheng N, Chen J, Li T, Liu W, Liu J, Chen H et al (2017) Abortifacient metapristone (RU486 derivative) interrupts CXCL12/CXCR4 axis for ovarian metastatic chemoprevention. *Mol Carcinog* 56(8):1896–1908
80. Zheng N, Chen J, Liu W, Liu J, Li T, Chen H et al (2017) Mifepristone inhibits ovarian cancer metastasis by intervening in SDF-1/CXCR4 chemokine axis. *Oncotarget* 8(35):59123–59135
81. Downward J (2003) Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 3(1):11–22
82. Vivanco I, Sawyers CL (2002) The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer* 2(7):489–501
83. Raj V, Bhadauria AS, Singh AK, Kumar U, Rai A, Keshari AK et al (2019) Novel 1,3,4-thiadiazoles inhibit colorectal cancer via blockade of IL-6/COX-2 mediated JAK2/STAT3 signals as evidenced through data-based mathematical modeling. *Cytokine* 118:144–159
84. Vogelstein B, Kinzler KW (2004) Cancer genes and the pathways they control. *Nat Med* 10(8):789–799
85. Wang D, Buchanan FG, Wang H, Dey SK, DuBois RN (2005) Prostaglandin E2 enhances intestinal adenoma growth via activation of the Ras-mitogen-activated protein kinase cascade. *Cancer Res* 65(5):1822–1829
86. Kaidi A, Qualtrough D, Williams AC, Paraskeva C (2006) Direct transcriptional up-regulation of cyclooxygenase-2 by hypoxia-inducible factor (HIF)-1 promotes colorectal tumor cell survival and enhances HIF-1 transcriptional activity during hypoxia. *Cancer Res* 66(13):6683–6691
87. Hattar K, Franz K, Ludwig M, Sibelius U, Wilhelm J, Lohmeyer J et al (2014) Interactions between neutrophils and non-small cell lung cancer cells: enhancement of tumor proliferation and inflammatory mediator synthesis. *Cancer Immunol Immunother* 63(12):1297–1306
88. Hull MA, Cuthbert RJ, Ko CWS, Scott DJ, Cartwright EJ, Hawcroft G et al (2017) Paracrine cyclooxygenase-2 activity by macrophages drives colorectal adenoma progression in the Apc (Min/+) mouse model of intestinal tumorigenesis. *Sci Rep* 7(1):6074
89. Esbona K, Yi Y, Saha S, Yu M, Van Doorn RR, Conklin MW et al (2018) The presence of cyclooxygenase 2, tumor-associated macrophages, and collagen alignment as prognostic markers for invasive breast carcinoma patients. *Am J Pathol* 188(3):559–573
90. Harris RE, Beebe-Donk J, Alshafie GA (2007) Cancer chemoprevention by cyclooxygenase 2 (COX-2) blockade: results of case control studies. *Subcell Biochem* 42:193–212
91. Massague J (2008) TGFbeta in cancer. *Cell* 134(2):215–230
92. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J et al (1995) Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* 268(5215):1336–1338
93. Tsujii M, DuBois RN (1995) Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell* 83(3):493–501
94. van de Wetering M, Sancho E, Verweij C, de Lau W, Oving I, Hurlstone A et al (2002) The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* 111(2):241–250
95. Castellone MD, Teramoto H, Williams BO, Druey KM, Gutkind JS (2005) Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. *Science* 310(5753):1504–1510
96. Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, Peters PJ et al (1998) Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat Genet* 19(4):379–383
97. Greenhough A, Smartt HJ, Moore AE, Roberts HR, Williams AC, Paraskeva C et al (2009) The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis* 30(3):377–386
98. Soto MS, O'Brien ER, Andreou K, Scrace SF, Zakaria R, Jenkinson MD et al (2016) Disruption of tumour-host communication by downregulation of LFA-1 reduces COX-2 and e-NOS expression and inhibits brain metastasis growth. *Oncotarget* 7(32):52375–52391
99. Singh B, Berry JA, Shohar A, Lucci A (2006) COX-2 induces IL-11 production in human breast cancer cells. *J Surg Res* 131(2):267–275
100. Hoing B, Kanaan O, Altenhoff P, Petri R, Thangavelu K, Schluter A et al (2018) Stromal versus tumoral inflammation differentially contribute to metastasis and poor survival in laryngeal squamous cell carcinoma. *Oncotarget* 9(9):8415–8426
101. Sorski L, Melamed R, Matzner P, Lavon H, Shaashua L, Rosenne E et al (2016) Reducing liver metastases of colon cancer in the context of extensive and minor surgeries through beta-adrenoceptors blockade and COX2 inhibition. *Brain Behav Immun* 58:91–98



102. Weinberg RA (2008) Mechanisms of malignant progression. *Carcinogenesis* 29(6):1092–1095
103. Gupta GP, Nguyen DX, Chiang AC, Bos PD, Kim JY, Nadal C et al (2007) Mediators of vascular remodelling co-opted for sequential steps in lung metastasis. *Nature* 446(7137):765–770
104. He TC, Chan TA, Vogelstein B, Kinzler KW (1999) PPARdelta is an APC-regulated target of nonsteroidal anti-inflammatory drugs. *Cell* 99(3):335–345
105. Sheng H, Shao J, Washington MK, DuBois RN (2001) Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. *J Biol Chem* 276(21):18075–18081
106. Tsujii M, Kawano S, DuBois RN (1997) Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci U S A* 94(7):3336–3340
107. Fenwick SW, Toogood GJ, Lodge JP, Hull MA (2003) The effect of the selective cyclooxygenase-2 inhibitor rofecoxib on human colorectal cancer liver metastases. *Gastroenterology* 125(3):716–729
108. Yao M, Kargman S, Lam EC, Kelly CR, Zheng Y, Luk P et al (2003) Inhibition of cyclooxygenase-2 by rofecoxib attenuates the growth and metastatic potential of colorectal carcinoma in mice. *Cancer Res* 63(3):586–592
109. Pai R, Nakamura T, Moon WS, Tarnawski AS (2003) Prostaglandins promote colon cancer cell invasion; signaling by cross-talk between two distinct growth factor receptors. *FASEB J* 17(12):1640–1647
110. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF (2003) Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 4(12):915–925
111. Pan J, Yang Q, Shao J, Zhang L, Ma J, Wang Y et al (2016) Cyclooxygenase-2 induced beta1-integrin expression in NSCLC and promoted cell invasion via the EPI/MAPK/E2F-1/FoxC2 signal pathway. *Sci Rep* 6:33823
112. Ko CJ, Lan SW, Lu YC, Cheng TS, Lai PF, Tsai CH et al (2017) Inhibition of cyclooxygenase-2-mediated matriptase activation contributes to the suppression of prostate cancer cell motility and metastasis. *Oncogene* 36(32):4597–4609
113. Majumder M, Landman E, Liu L, Hess D, Lala PK (2015) COX-2 elevates oncogenic miR-526b in breast cancer by EP4 activation. *Mol Cancer Res* 13(6):1022–1033
114. Xian X, Huang L, Zhang B, Wu C, Cui J, Wang Z (2016) WIN 55,212-2 inhibits the epithelial mesenchymal transition of gastric cancer cells via COX-2 signals. *Cell Physiol Biochem* 39(6):2149–2157
115. Williams CS, Tsujii M, Reese J, Dey SK, DuBois RN (2000) Host cyclooxygenase-2 modulates carcinoma growth. *J Clin Invest* 105(11):1589–1594
116. Hull MA, Faluyi OO, Ko CW, Holwell S, Scott DJ, Cuthbert RJ et al (2006) Regulation of stromal cell cyclooxygenase-2 in the ApcMin/+ mouse model of intestinal tumorigenesis. *Carcinogenesis* 27(3):382–391
117. Kerr JFR, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26:239–257
118. Adams JM (2003) Ways of dying: multiple pathways to apoptosis. *Genes Dev* 17(20):2481–2495
119. Green DR (1998) Apoptotic pathways: the roads to ruin. *Cell* 94(6):695–698
120. Thompson CB (1995) Apoptosis in the pathogenesis and treatment of disease. *Science* 267:1456–1462
121. Green DR, Evan GI (2002) A matter of life and death. *Cancer Cell* 1(1):19–30
122. Pelengaris S, Khan M, Evan GI (2002) Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. *Cell* 109(3):321–334
123. Gungor H, Ilhan N, Eroksuz H (2018) The effectiveness of cyclooxygenase-2 inhibitors and evaluation of angiogenesis in the model of experimental colorectal cancer. *Biomed Pharmacother* 102:221–229
124. Hosseini F, Mahdian-Shakib A, Jadidi-Niaragh F, Enderami SE, Mohammadi H, Hemmatzadeh M et al (2018) Anti-inflammatory and anti-tumor effects of alpha-l-guluronic acid (G2013) on cancer-related inflammation in a murine breast cancer model. *Biomed Pharmacother* 98:793–800
125. Todoric J, Antonucci L, Karin M (2016) Targeting inflammation in cancer prevention and therapy. *Cancer Prev Res (Phila)* 9(12):895–905
126. Chen W, Bai L, Wang X, Xu S, Belinsky SA, Lin Y (2010) Acquired activation of the Akt/cyclooxygenase-2/Mcl-1 pathway renders lung cancer cells resistant to apoptosis. *Mol Pharmacol* 77(3):416–423
127. Krysan K, Dalwadi H, Sharma S, Pold M, Dubinett S (2004) Cyclooxygenase 2-dependent expression of survivin is critical for apoptosis resistance in non-small cell lung cancer. *Cancer Res* 64(18):6359–6362
128. Janakiraman H, House RP, Talwar S, Courtney SM, Hazard ES, Hardiman G et al (2017) Repression of caspase-3 and RNA-binding protein HuR cleavage by cyclooxygenase-2 promotes drug resistance in oral squamous cell carcinoma. *Oncogene* 36(22):3137–3148
129. Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN (1998) Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. *Cancer Res* 58(2):362–366
130. Pozzi A, Yan X, Macias-Perez I, Wei S, Hata AN, Breyer RM et al (2004) Colon carcinoma cell growth is associated with prostaglandin E2/EP4 receptor-evoked ERK activation. *J Biol Chem* 279(28):29797–29804
131. Tessner TG, Muhale F, Riehl TE, Anant S, Stenson WF (2004) Prostaglandin E2 reduces radiation-induced epithelial apoptosis through a mechanism involving AKT activation and bax translocation. *J Clin Invest* 114(11):1676–1685

132. Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS (2002) Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. *Nat Med* 8(3):289–293
133. Buchanan FG, Wang D, Bargiacchi F, DuBois RN (2003) Prostaglandin E2 regulates cell migration via the intracellular activation of the epidermal growth factor receptor. *J Biol Chem* 278(37):35451–35457
134. Leone V, di Palma A, Ricchi P, Acquaviva F, Giannouli M, Di Prisco AM et al (2007) PGE2 inhibits apoptosis in human adenocarcinoma Caco-2 cell line through Ras-PI3K association and cAMP-dependent kinase A activation. *Am J Physiol Gastrointest Liver Physiol* 293(4):G673–G681
135. Chin YT, Wei PL, Ho Y, Nana AW, Changou CA, Chen YR et al (2018) Thyroxine inhibits resveratrol-caused apoptosis by PD-L1 in ovarian cancer cells. *Endocr Relat Cancer* 25(5):533–545
136. Lang S, Picu A, Hofmann T, Andratschke M, Mack B, Moosmann A et al (2006) COX-inhibitors relieve the immunosuppressive effect of tumor cells and improve functions of immune effectors. *Int J Immunopathol Pharmacol* 19(2):409–419
137. Miao J, Lu X, Hu Y, Piao C, Wu X, Liu X et al (2017) Prostaglandin E2 and PD-1 mediated inhibition of antitumor CTL responses in the human tumor micro-environment. *Oncotarget* 8(52):89802–89810
138. Li Q, Liu L, Zhang Q, Liu S, Ge D, You Z (2014) Interleukin-17 indirectly promotes M2 macrophage differentiation through stimulation of COX-2/PGE2 pathway in the cancer cells. *Cancer Res Treat* 46(3):297–306
139. Dubey P, Shrivastava R, Tripathi C, Jain NK, Tewari BN, Lone MU et al (2014) Cyclooxygenase-2 inhibition attenuates hypoxic cancer cells induced m2-polarization of macrophages. *Cell Mol Biol (Noisy-le-Grand)* 60(3):10–15
140. Holt D, Ma X, Kundu N, Fulton A (2011) Prostaglandin E(2) (PGE (2)) suppresses natural killer cell function primarily through the PGE(2) receptor EP4. *Cancer Immunol Immunother* 60(11):1577–1586
141. Gobel C, Breitenbuecher F, Kalkavan H, Hahnel PS, Kasper S, Hoffarth S et al (2014) Functional expression cloning identifies COX-2 as a suppressor of antigen-specific cancer immunity. *Cell Death Dis* 5:e1568
142. Okano M, Sugata Y, Fujiwara T, Matsumoto R, Nishibori M, Shimizu K et al (2006) E prostanoid 2 (EP2)/EP4-mediated suppression of antigen-specific human T-cell responses by prostaglandin E2. *Immunology* 118(3):343–352
143. Gualde N, Harizi H (2004) Prostanoids and their receptors that modulate dendritic cell-mediated immunity. *Immunol Cell Biol* 82(4):353–360
144. Mougiakakos D, Johansson CC, Trocme E, All-Ericsson C, Economou MA, Larsson O et al (2010) Intratumoral forkhead box P3-positive regulatory T cells predict poor survival in cyclooxygenase-2-positive uveal melanoma. *Cancer* 116(9):2224–2233
145. Yuan XL, Chen L, Li MX, Dong P, Xue J, Wang J et al (2010) Elevated expression of Foxp3 in tumor-infiltrating Treg cells suppresses T-cell proliferation and contributes to gastric cancer progression in a COX-2-dependent manner. *Clin Immunol* 134(3):277–288
146. Hori S, Nomura T, Sakaguchi S (2003) Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299(5609):1057–1061
147. Mahic M, Yaqub S, Johansson CC, Tasken K, Aandahl EM (2006) FOXP3+CD4+CD25+ adaptive regulatory T cells express cyclooxygenase-2 and suppress effector T cells by a prostaglandin E2-dependent mechanism. *J Immunol* 177(1):246–254
148. Martinet L, Jean C, Dietrich G, Fournie JJ, Poupot R (2010) PGE2 inhibits natural killer and gamma delta T cell cytotoxicity triggered by NKR and TCR through a cAMP-mediated PKA type I-dependent signaling. *Biochem Pharmacol* 80(6):838–845
149. Markosyan N, Chen EP, Ndong VN, Yao Y, Serner CJ, Chodosh LA et al (2011) Deletion of cyclooxygenase 2 in mouse mammary epithelial cells delays breast cancer onset through augmentation of type 1 immune responses in tumors. *Carcinogenesis* 32(10):1441–1449
150. Harizi H, Juzan M, Pitard V, Moreau JF, Gualde N (2002) Cyclooxygenase-2-issued prostaglandin e(2) enhances the production of endogenous IL-10, which down-regulates dendritic cell functions. *J Immunol* 168(5):2255–2263
151. Harizi H, Grosset C, Gualde N (2003) Prostaglandin E2 modulates dendritic cell function via EP2 and EP4 receptor subtypes. *J Leukoc Biol* 73(6):756–763
152. Harris SG, Padilla J, Koumas L, Ray D, Phipps RP (2002) Prostaglandins as modulators of immunity. *Trends Immunol* 23(3):144–150
153. Harizi H, Gualde N (2005) The impact of eicosanoids on the crosstalk between innate and adaptive immunity: the key roles of dendritic cells. *Tissue Antigens* 65(6):507–514
154. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB et al (1998) Cyclooxygenase in biology and disease. *FASEB J* 12(12):1063–1073
155. Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner BM et al (2000) Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. *Cancer Res* 60(5):1306–1311
156. Kirschenbaum A, Liu X, Yao S, Levine AC (2001) The role of cyclooxygenase-2 in prostate cancer. *Urology* 58(2 Suppl 1):127–131
157. Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. *Nat Med* 9(6):669–676
158. Seno H, Oshima M, Ishikawa TO, Oshima H, Takaku K, Chiba T et al (2002) Cyclooxygenase 2- and prostaglandin E(2) receptor EP(2)-dependent angiogenesis in Apc(Delta716) mouse intestinal polyps. *Cancer Res* 62(2):506–511

159. Dormond O, Foletti A, Paroz C, Ruegg C (2001) NSAIDs inhibit alpha V beta 3 integrin-mediated and Cdc42/Rac-dependent endothelial-cell spreading, migration and angiogenesis. *Nat Med* 7(9):1041–1047
160. Fukuda R, Kelly B, Semenza GL (2003) Vascular endothelial growth factor gene expression in colon cancer cells exposed to prostaglandin E2 is mediated by hypoxia-inducible factor 1. *Cancer Res* 63(9):2330–2334
161. Salcedo R, Zhang X, Young HA, Michael N, Wasserman K, Ma WH et al (2003) Angiogenic effects of prostaglandin E2 are mediated by up-regulation of CXCR4 on human microvascular endothelial cells. *Blood* 102(6):1966–1977
162. Wang D, Wang H, Brown J, Daikoku T, Ning W, Shi Q et al (2006) CXCL1 induced by prostaglandin E2 promotes angiogenesis in colorectal cancer. *J Exp Med* 203(4):941–951
163. Carmeliet P (2000) Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 6(4):389–395
164. Lee A, Frischer J, Serur A, Huang J, Bae JO, Kornfield ZN et al (2006) Inhibition of cyclooxygenase-2 disrupts tumor vascular mural cell recruitment and survival signaling. *Cancer Res* 66(8):4378–4384
165. Szveda M, Rychlik A, Babinska I, Pomianowski A (2019) Significance of cyclooxygenase-2 in oncogenesis. *J Vet Res* 63(2):215–224
166. Hirschi KK, D'Amore PA (1996) Pericytes in the microvasculature. *Cardiovasc Res* 32(4):687–698
167. Diaz-Flores L, Gutierrez R, Varela H, Rancel N, Valladares F (1991) Microvascular pericytes: a review of their morphological and functional characteristics. *Histol Histopathol* 6(2):269–286
168. Birbrair A (2018) Pericyte biology: development, homeostasis, and disease. *Adv Exp Med Biol* 1109:1–3
169. Erber R, Thurnher A, Katsen AD, Groth G, Kerger H, Hammes HP et al (2004) Combined inhibition of VEGF and PDGF signaling enforces tumor vessel regression by interfering with pericyte-mediated endothelial cell survival mechanisms. *FASEB J* 18(2):338–340
170. Frischer JS, Huang J, Serur A, Kadenhe-Chiweshe A, McCrudden KW, O'Toole K et al (2004) Effects of potent VEGF blockade on experimental Wilms tumor and its persisting vasculature. *Int J Oncol* 25(3):549–553
171. Huang J, Soffer SZ, Kim ES, McCrudden KW, Huang J, New T et al (2004) Vascular remodeling marks tumors that recur during chronic suppression of angiogenesis. *Mol Cancer Res* 2(1):36–42
172. Birbrair A, Zhang T, Wang ZM, Messi ML, Olson JD, Mintz A et al (2014) Type-2 pericytes participate in normal and tumoral angiogenesis. *Am J Physiol Cell Physiol* 307(1):C25–C38
173. Yang X, Sheares KK, Davie N, Upton PD, Taylor GW, Horsley J et al (2002) Hypoxic induction of cox-2 regulates proliferation of human pulmonary artery smooth muscle cells. *Am J Respir Cell Mol Biol* 27(6):688–696
174. Inoue H, Taba Y, Miwa Y, Yokota C, Miyagi M, Sasaguri T (2002) Transcriptional and posttranscriptional regulation of cyclooxygenase-2 expression by fluid shear stress in vascular endothelial cells. *Arterioscler Thromb Vasc Biol* 22(9):1415–1420
175. Subbaramaiah K, Dannenberg AJ (2003) Cyclooxygenase 2: a molecular target for cancer prevention and treatment. *Trends Pharmacol Sci* 24(2):96–102
176. Csiki I, Yanagisawa K, Haruki N, Nadaf S, Morrow JD, Johnson DH et al (2006) Thioredoxin-1 modulates transcription of cyclooxygenase-2 via hypoxia-inducible factor-1alpha in non-small cell lung cancer. *Cancer Res* 66(1):143–150
177. Sheng H, Shao J, Hooton EB, Tsujii M, DuBois RN, Beauchamp RD (1997) Cyclooxygenase-2 induction and transforming growth factor beta growth inhibition in rat intestinal epithelial cells. *Cell Growth Differ: the molecular biology journal of the American Association for Cancer Research* 8(4):463–470
178. Guo YS, Cheng JZ, Jin GF, Gutkind JS, Hellmich MR, Townsend CM Jr (2002) Gastrin stimulates cyclooxygenase-2 expression in intestinal epithelial cells through multiple signaling pathways. Evidence for involvement of ERK5 kinase and transactivation of the epidermal growth factor receptor. *J Biol Chem* 277(50):48755–48763
179. Jones MK, Sasaki E, Halter F, Pai R, Nakamura T, Arakawa T et al (1999) HGF triggers activation of the COX-2 gene in rat gastric epithelial cells: action mediated through the ERK2 signaling pathway. *FASEB J* 13(15):2186–2194
180. Howe LR, Subbaramaiah K, Chung WJ, Dannenberg AJ, Brown AM (1999) Transcriptional activation of cyclooxygenase-2 in Wnt-1-transformed mouse mammary epithelial cells. *Cancer Res* 59(7):1572–1577
181. Howe LR, Crawford HC, Subbaramaiah K, Hassell JA, Dannenberg AJ, Brown AM (2001) PEA3 is up-regulated in response to Wnt1 and activates the expression of cyclooxygenase-2. *J Biol Chem* 276(23):20108–20115
182. Araki Y, Okamura S, Hussain SP, Nagashima M, He P, Shiseki M et al (2003) Regulation of cyclooxygenase-2 expression by the Wnt and ras pathways. *Cancer Res* 63(3):728–734
183. Theodoraki MN, Hoffmann TK, Whiteside TL (2018) Separation of plasma-derived exosomes into CD3(+) and CD3(−) fractions allows for association of immune cell and tumour cell markers with disease activity in HNSCC patients. *Clin Exp Immunol* 192(3):271–283
184. Xu Y, Yang X, Wang T, Yang L, He YY, Miskimins K et al (2018) Knockdown delta-5-desaturase in breast cancer cells that overexpress COX-2 results in inhibition of growth, migration and invasion via a dihomo-gamma-linolenic acid peroxidation dependent mechanism. *BMC Cancer* 18(1):330

185. Qiu HY, Wang PF, Li Z, Ma JT, Wang XM, Yang YH et al (2016) Synthesis of dihydropyrazole sulphonamide derivatives that act as anti-cancer agents through COX-2 inhibition. *Pharmacol Res* 104:86–96
186. Kirk J, Shah N, Noll B, Stevens CB, Lawler M, Mougeot FB et al (2018) Text mining-based in silico drug discovery in oral mucositis caused by high-dose cancer therapy. *Support Care Cancer* 26(8):2695–2705
187. Ikeya S, Sakabe JI, Yamada T, Naito T, Tokura Y (2018) Voriconazole-induced photocarcinogenesis is promoted by aryl hydrocarbon receptor-dependent COX-2 upregulation. *Sci Rep* 8(1):5050
188. Glinghammar B, Inoue H, Rafter JJ (2002) Deoxycholic acid causes DNA damage in colonic cells with subsequent induction of caspases, COX-2 promoter activity and the transcription factors NF-kB and AP-1. *Carcinogenesis* 23(5):839–845
189. Wang J, Cho NL, Zauber AG, Hsu M, Dawson D, Srivastava A et al (2018) Chemopreventive efficacy of the Cyclooxygenase-2 (Cox-2) inhibitor, celecoxib, is predicted by adenoma expression of Cox-2 and 15-PGDH. *Cancer Epidemiol Biomarkers Prev: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 27:728
190. Ng K, Meyerhardt JA, Chan AT, Sato K, Chan JA, Niedzwiecki D et al (2015) Aspirin and COX-2 inhibitor use in patients with stage III colon cancer. *J Natl Cancer Inst* 107(1):345
191. Kargman SL, O'Neill GP, Vickers PJ, Evans JF, Mancini JA, Jothy S (1995) Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. *Cancer Res* 55(12):2556–2559
192. Zhu LL, Xu LC, Chen Y, Zhou Q, Zeng S (2012) Poor awareness of preventing aspirin-induced gastrointestinal injury with combined protective medications. *World J Gastroenterol* 18(24):3167–3172
193. Cao Y, Prescott SM (2002) Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. *JCell Physiol* 190(3):279–286
194. Punganuru SR, Madala HR, Mikelis CM, Dixit A, Arutla V, Srivenugopal KS (2018) Conception, synthesis, and characterization of a rofecoxib-combretastatin hybrid drug with potent cyclooxygenase-2 (COX-2) inhibiting and microtubule disrupting activities in colon cancer cell culture and xenograft models. *Oncotarget* 9(40):26109–26129
195. Hudson M, Richard H, Pilote L (2007) Parabolas of medication use and discontinuation after myocardial infarction--are we closing the treatment gap? *Pharmacoepidemiol Drug Saf* 16(7):773–785
196. Russell RI (2001) Non-steroidal anti-inflammatory drugs and gastrointestinal damage-problems and solutions. *Postgrad Med J* 77(904):82–88
197. Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R et al (2003) A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *N Engl J Med* 348(10):883–890
198. Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K et al (2006) Celecoxib for the prevention of sporadic colorectal adenomas. *N Engl J Med* 355(9):873–884
199. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K et al (2005) Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med* 352(11):1092–1102
200. Arber N, Eagle CJ, Spicak J, Racz I, Dite P, Hajer J et al (2006) Celecoxib for the prevention of colorectal adenomatous polyps. *N Engl J Med* 355(9):885–895
201. Martinez JA, Yang J, Wertheim BC, Roe DJ, Schriever A, Lance P et al (2018) Celecoxib use and circulating oxylipins in a colon polyp prevention trial. *PLoS One* 13(4):e0196398
202. Harris RE (2009) Cyclooxygenase-2 (cox-2) blockade in the chemoprevention of cancers of the colon, breast, prostate, and lung. *Inflammopharmacology* 17(2):55–67
203. Buchanan FG, Holla V, Katkuri S, Matta P, DuBois RN (2007) Targeting cyclooxygenase-2 and the epidermal growth factor receptor for the prevention and treatment of intestinal cancer. *Cancer Res* 67(19):9380–9388
204. Reckamp KL, Krysan K, Morrow JD, Milne GL, Newman RA, Tucker C et al (2006) A phase I trial to determine the optimal biological dose of celecoxib when combined with erlotinib in advanced non-small cell lung cancer. *Clin Cancer Res* 12(11 Pt 1):3381–3388
205. Barry M, Cahill RA, Roche-Nagle G, Neilan TG, Treumann A, Harmey JH et al (2009) Neoplasms escape selective COX-2 inhibition in an animal model of breast cancer. *Ir J Med Sci* 178(2):201–208
206. Anderson GD, Keys KL, De Ciechi PA, Masferrer JL (2009) Combination therapies that inhibit cyclooxygenase-2 and leukotriene synthesis prevent disease in murine collagen induced arthritis. *Inflamm Res* 58(2):109–117
207. Falandry C, Canney PA, Freyer G, Dirix LY (2009) Role of combination therapy with aromatase and cyclooxygenase-2 inhibitors in patients with metastatic breast cancer. *Ann Oncol* 20(4):615–620
208. Duan DP, Dang XQ, Wang KZ, Wang YP, Zhang H, You WL (2012) The cyclooxygenase-2 inhibitor NS-398 inhibits proliferation and induces apoptosis in human osteosarcoma cells via downregulation of the survivin pathway. *Oncol Rep* 28(5):1693–1700
209. Mullins MN, Lana SE, Dernel WS, Ogilvie GK, Withrow SJ, Ehrhart EJ (2004) Cyclooxygenase-2 expression in canine appendicular osteosarcomas. *J Vet Intern Med* 18(6):859–865
210. Naruse T, Nishida Y, Hosono K, Ishiguro N (2006) Meloxicam inhibits osteosarcoma growth, invasiveness and metastasis by COX-2-dependent and independent routes. *Carcinogenesis* 27(3):584–592

211. Urakawa H, Nishida Y, Naruse T, Nakashima H, Ishiguro N (2009) Cyclooxygenase-2 overexpression predicts poor survival in patients with high-grade extremity osteosarcoma: a pilot study. *Clin Orthop Relat Res* 467(11):2932–2938
212. Arjona-Sanchez A, Ruiz-Rabelo J, Perea MD, Vazquez R, Cruz A, Munoz Mdel C et al (2010) Effects of capecitabine and celecoxib in experimental pancreatic cancer. *Pancreatology* 10(5):641–647
213. Howe LR, Subbaramaiah K, Patel J, Masferrer JL, Deora A, Hudis C et al (2002) Celecoxib, a selective cyclooxygenase 2 inhibitor, protects against human epidermal growth factor receptor 2 (HER-2)/neu-induced breast cancer. *Cancer Res* 62(19):5405–5407
214. Dandekar DS, Lopez M, Carey RI, Lokeshwar BL (2005) Cyclooxygenase-2 inhibitor celecoxib augments chemotherapeutic drug-induced apoptosis by enhancing activation of caspase-3 and -9 in prostate cancer cells. *Int J Cancer* 115(3):484–492