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Anti-tumor activity of non-steroidal anti-inflammatory drugs: Cyclooxygenase-independent targets

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are used extensively for analgesic and antipyretic treatments. In addition, NSAIDs reduce the risk and mortality to several cancers. Their mechanisms in anti-tumorigenesis are not fully understood, but both cyclooxygenase (COX)-dependent and -independent pathways play a role. We and others have been interested in elucidating molecular targets of NSAID-induced apoptosis. In this review, we summarize updated literature regarding cellular and molecular targets modulated by NSAIDs. Among those NSAIDs, sulindac sulfide and tolfenamic acid are emphasized in this review because these two drugs have been well investigated for their anti-tumorigenic activity in many different types of cancer.

Keywords

NSAIDs; COX; sulindac sulfide; tolfenamic acid; NAG-1

1. Introduction

Despite advancements in modern medicine, cancer is a major cause of death worldwide, with a death toll of 7.6 million or about 13% of all deaths per year [1]. This along with increasing life expectancies in the developed world produces more chances of acquiring cancer and makes research into the mechanisms of cancer prevention a high priority.

Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of drugs that have been utilized for their analgesic and antipyretic effects for centuries. In more recent history, NSAIDs have also been widely used and studied for their anti-tumorigenic and chemopreventive properties. The classical pathway of action for NSAIDs is blocking the generation of

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Conflict of Interest Statement

None

prostaglandins from arachidonic acid (AA) via inhibition of cyclooxygenase-1 and -2 (COX-1 and -2) activities. COX-1 is present and expressed constitutively in most tissues, whereas COX-2 expression is usually transient and can be rapidly induced by cytokines, lipopolysaccharide (LPS), phorbol myristate acetate (PMA), or growth factors [2–4]. The inhibition of both COX-1 and CO-2 by conventional NSAIDs such as aspirin, ibuprofen, and naproxen, could have negative consequences due to inhibition of prostaglandin (PG) synthesis. Particularly, PGE₂ has a cytoprotective effects on the gastric mucosa and renal epithelial cells [5]. Therefore, the prolonged use of conventional NSAIDs that inhibit both COX-1 and COX-2 can lead to side effects including gastrointestinal tract bleeding and kidney failure, which may be attributable to COX-1 inhibition. In addition, a recent clinical trial of familial adenomatous polyposis (FAP) patients show a three-fold increase in the risk of cardio-toxicity from treatment with the COX-2 inhibitor celecoxib [6, 7]. These side effects are part of the motivation that drives research into the COX-independent mechanisms of NSAIDs. Among NSAIDs, tolfenamic acid (TA) and sulindac sulfide (SS) have been extensively investigated for their anti-tumorigenic properties because these two drugs exhibit better anti-cancer activity than many other NSAIDs [8–10]. There is still much work to be done elucidating the pathways of COX-independent activity of NSAIDs, and this review primarily focuses on intracellular molecular targets that are affected by NSAIDs.

2. Transcription factors

NSAIDs have been shown to affect a wide variety of transcription factors, including EGR-1, ATF3, ATF4, Sp1, CHOP, NF- κ B, β -catenin/TCF, and Smad. These transcription factors have been identified as either pro-tumorigenic or anti-tumorigenic, depending on cell context and types. In this section, we summarize how these transcription factors are altered by NSAIDs as related to tumorigenesis (Fig. 1).

2.1. EGR-1

The zinc-finger DNA binding protein early growth response-1 (EGR-1) is involved in many activities including differentiation and tumorigenesis. EGR-1 acts as a tumor suppressor in mouse two-step skin carcinogenesis using EGR-1 knock-out mice, and binds to the p53 promoter region both *in vitro* and *in vivo* [11]. Furthermore, EGR-1 plays an important role in up-regulating the expression of the anti-tumorigenic/pro-apoptotic protein NSAID activated gene-1 (NAG-1) and the anti-angiogenic protein thrombospondin-1 (TSP-1) in colorectal and lung cancer cells, respectively, during sulindac sulfide (SS) treatment [12, 13]. NSAIDs up-regulate EGR-1, which binds to the promoter regions of NAG-1 and TSP-1. This up-regulation has been confirmed *in vivo*, using a Sprague-Dawley rat model for early colorectal tumorigenesis, which uses the colon-specific pro-carcinogen 1,2-dimethylhydrazine dihydrochloride (DMH) [14]. EGR-1 protein expression is induced in rats treated with sulindac or celecoxib, along with less tumor formation compared to control rats [14]. Another conventional NSAID, TA, also induces EGR-1 at the transcription level via enhancement of epithelium-specific ETS transcription factor-1 (ESE-1) nuclear translocation, which leads to activation of apoptosis in colorectal cancer cells [15]. In addition, natural anti-cancer compounds such as resveratrol can induce apoptosis via EGR-1 induction [16]. However, depending on the cell type and conditions, EGR-1 can also play a negative role in tumorigenesis. In androgen-independent prostate cancer, EGR-1 is required for mediating CXCL5/ENA78's oncogenic activity in cell growth, migration, and epithelial-to-mesenchymal transition (EMT) [17]. Thus, EGR-1 plays a complex role, acting through many pathways including anti- and/or pro-tumorigenic activity. Therefore, although NSAIDs increase EGR-1 expression, further study of EGR-1 function in tumorigenesis is needed.

2.2. ATF3

ATF3 is a cyclic adenosine monophosphate (cAMP)-dependent transcription factor that is a member of the basic region leucine zipper (bZIP) family [18]. ATF3 is induced by the conventional NSAIDs SS and TA in human colorectal cancer cells [19]. Treatment of colorectal cancer cells (CRC) with TA increases ATF3 protein expression, which is highly associated with apoptosis induction. Furthermore, ATF2 phosphorylation by TA increases ATF3 promoter activity via direct binding of phospho-ATF2 in the ATF3 promoter [19].

Both ATF3 over-expression and SS treatment down-regulate metastasis-associated protein (MTA1) and β -catenin in HCT-116 cells [20]. Thus, ATF3 has been implicated in several anti-tumorigenic pathways and represents one explanation for NSAIDs' COX-independent activity.

2.3. ATF4

ATF4 is another bZIP transcriptional factor that is usually up-regulated under cellular stress by a translational control mechanism [21]. In addition, hypoxia can induce ATF4 up-regulation by increasing its stability [22]. Various NSAIDs have been shown to trigger endoplasmic reticulum (ER) stress response, resulting in the induction of ATF4, which is involved in NSAID-induced apoptosis in cultured guinea-pig gastric mucosal cells [23]. Moreover, celecoxib activates the Ca^{2+} -dependent PERK-eIF2a-ATF4 pathway, which causes apoptosis through up-regulation of PUMA expression in human gastric carcinoma cell lines [24]. On the other hand, celecoxib-induced ATF4 expression could contribute to the up-regulation of glucose-regulated protein 78 (GRP78), an ER chaperone, and S100P, a member of the S100 family of EF-hand Ca^{2+} -binding proteins, both of which may partially decrease the potential capability of celecoxib against cancer [25, 26]. More recently, we also showed evidence that ATF4 could play a role in apoptosis induced by TA in CRC cells [9]. Collectively, NSAIDs increase ATF4 expression via inducing ER stress, which may play complicated roles in gastric tumor suppression, and possibly drug resistance as well.

2.4 CHOP

The C/EBP-homologous protein (CHOP) is an ER stress response protein and member of the bZIP transcription factor family; CHOP inhibits CCAAT/enhancer-binding protein (C/EBP) and liver-enriched transcriptional activator protein (LAP) DNA-binding activity [27]. CHOP mRNA and protein levels are both elevated by SS treatment in CRC cells [28]. Celecoxib also induces CHOP protein expression in malignant glioma cells, whereas SS does not unless co-treated with carbonyl cyanide 4-(trifluoromethoxy)-phenylhydrazone (FCCP) [29]. FCCP is a protonophore which uncouples oxidative phosphorylation causing the loss of mitochondrial membrane potential; FCCP does not induce CHOP protein by itself in malignant glioma cells [29, 30]. These results suggest that mitochondrial disruption accompanied by ER stress response is needed to induce CHOP expression in malignant glioma cells. Subsequently, recent study from our group suggests that TA affects ER stress, thereby enhancing apoptosis via CHOP activity in human CRC cells [9]. Interestingly, pranoprofen suppresses ER stress-induced CHOP expression, likely through the inhibition of XBP-1 splicing in glial cells [31]. Taken together, these results suggest that there are complicated mechanisms involved in NSAID-induced CHOP expression, and that these mechanisms are dependent on cell context and chemical structure.

2.5. Sp

Specificity proteins (Sp) are zinc-finger transcription factors that are members of the Sp/KLF sub-family [32]. Sp proteins bind to the GC and/or GT boxes of many promoters. There are several Sp proteins, including Sp1, Sp2, Sp3, Sp4, Sp5, Sp6/KLF14, Sp7, and

Sp8. This section focuses on Sp1, Sp3, and Sp4, which are phylogenetically more similar to one another than to the other Sp proteins [33, 34].

Sp1 is normally responsible for controlling various housekeeping genes; however, Sp1 has been linked to the regulation of tumorigenesis [35]. Sp proteins contribute to the proliferation of metastatic tumor phenotypes, and thus overexpression of these transcription factors is a negative survival prognostic factor in many human cancers [35].

TA has been shown to decrease Sp1, Sp3, and Sp4 protein expression as well as vascular endothelial growth factor receptor-1 (VEGFR-1) protein expression in pancreatic cancer cells [36]. Interestingly, small interfering RNA (siRNA) for Sp1, Sp3, and Sp4 also lead to decreased VEGFR-1 [36]. Thus, TA decreases this important angiogenic factor via regulation of Sp proteins. These results are supported by nude mouse data that show that TA reduces Sp1, Sp3, and Sp4 protein expression *in vivo* as well [8]. TA has also been shown to decrease Sp1, Sp3, and Sp4 in rhabdomyosarcoma (RMS) cells and in lung cancer cells both *in vitro* as well as in mouse models [37, 38]. COX-2-preferential NSAIDs, including celecoxib, also down-regulate Sp1 and Sp4 activity in human CRC cells; however, Sp3 protein expression is unchanged in this case [39]. This action was shown to be through COX-2-independent activation of Sp1 and Sp4 proteasomal degradation [39]. Recently, Dr. Safe's group has further shown that TA and the novel nitric oxide (NO) chimera containing NSAID ethyl 2-((2,3-bis(nitrooxy)propyl) disulfanyl) benzoate (GT-094) down-regulate Sp1, Sp3, and Sp4 in human CRC cells both *in vitro* and in a mouse xenograft model, leading to decreased expression of VEGF/VEGFR-1, cyclin D1, hepatocyte growth factor receptor, survivin, Bcl-2, and NF- κ B [40, 41]. Aspirin and sodium salicylate have also recently been shown to decrease Sp1, Sp3, and Sp4 in a caspase-dependent manner in human CRC cells *in vitro* as well as in a mouse model [42]. Taken together, these data show that NSAIDs down-regulate Sp proteins in several different cancers including pancreas, lung, rhabdomyosarcoma, and colon cancers. The consistency of these results makes NSAID action through Sp protein regulation a promising pathway.

2.6. NF- κ B

The roles of NF- κ B in cancer progression and anti-cancer therapeutics are complex. There is evidence to suggest that NF- κ B activation is associated with increased survival of cancer cells and resistance to chemotherapy; thus inhibition of NF- κ B activity is regarded as a target for anti-cancer therapy [43, 44]. Aspirin inhibits the NF- κ B pathway by interaction with IKK β [45]. In an *in vivo* model using male Sprague-Dawley rats given DMH to produce early stages of CRC, SS and celecoxib inhibited DMH-mediated I κ B β down-regulation and DMH-mediated IKK β up-regulation, followed by interfering nuclear localization of NF- κ B [46]. Through *in silico* 3D crystal structure modeling, this study also suggests that both SS and celecoxib directly bind to NF- κ B docking sites [46]. However, NF- κ B activation is capable of promoting a pro-apoptotic response under different circumstances [47, 48]. SS and TA induce NF- κ B activity and apoptosis in human CRC cells [49, 50]. Thus, further study is needed to elucidate NF- κ B's role in tumorigenesis, although NSAIDs could activate or inhibit NF- κ B activity.

2.7. β -catenin/TCF

The β -catenin/TCF signaling pathway plays a key role in regulation of tumorigenesis. In colorectal tumor cells, loss of APC function results in increased levels of β -catenin. The elevated levels of β -catenin promote its interaction with transcription factors of the TCF/Lef family and translocation of the β -catenin/TCF-complex to the nucleus, where it modulates transcription of downstream genes involved in pro-tumorigenesis. Both aspirin and indomethacin suppress β -catenin target genes by modulating TCF activity [51]. Nuclear β -

catenin content is also decreased by treatment with several other NSAIDs [52, 53]. However, this effect of NSAIDs on β -catenin suppression is dependent on PPAR γ and RXR α expression [54]. Thus, NSAIDs down-regulate β -catenin expression at the transcriptional and/or translational levels, leading to its reduced nuclear translocation. Furthermore, it is speculated that NSAIDs may be useful as anti-metastatic drugs by inhibiting β -catenin activity, leading to inhibition of metastatic protein S100A4 transcription [55].

2.8. Smad

Smad transcription factors serve as vital mediators in TGF- β signaling; these transcription factors normally form heteromeric complexes and then translocate into the nucleus where they activate target gene expression together with co-activators and/or suppressors [56]. Smad signaling has tumor-suppressive effects in the early stages of tumorigenesis; however, paradoxically, Smad signaling contributes to metastasis in advanced stage tumors. There are a large number of proteins targeted by the Smad pathway, over half of which may be involved in cancer cell invasion and migration [57]. Therefore, disruption of Smad signaling in late-stage tumorigenesis could be a potential cancer therapeutic strategy. The NSAID, 5-aminosalicylic acid (5-ASA) has been reported to suppress TGF- β -driven Smad2/3 phosphorylation and subsequently inhibit translocation of Smad2/3 into nucleus in CRC cells [58]. Moreover, the intake of aspirin seems to modulate Smad gene variation in colorectal tumors [59]. Our recent finding also shows that TA inhibits TGF- β -induced Smad phosphorylation via the ERK MAP kinase pathway in various cancer cells [60]. More importantly, TA had the best inhibitory activity of Smad phosphorylation compared with other tested NSAIDs. These data suggest that the interference of Smad signaling could be a novel mechanism of NSAID anti-tumor activity.

3. Cell signaling proteins

Cell signaling proteins are a diverse group of proteins that perform a wide variety of functions. Some are adhesion modules responsible for cell-cell binding while others are responsible for anchoring the plasma membrane to the actin cytoskeleton. Cell surface proteins can act as receptors for paracrine and autocrine cell signaling and are responsible for signal transduction to cytoplasmic signaling proteins and even translocation into the nucleus to affect transcription. These proteins are highly studied in cancer research due to their potential prognostic value as cancer markers and the promising chemotherapy potential of cancer cell-specific drug delivery (Fig. 2).

3.1 EGFR

Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that is mutated and/or over-active in a high percentage of cancers. EGFR signaling can lead to cell proliferation through the Erk1/2 pathway and cell survival, invasion, and angiogenesis through the AKT pathway. Sulindac metabolites, SS and sulindac sulfone, down-regulate EGFR signaling by inhibiting activation and/or expression of EGFR in CRC cell lines [61]. The dual NSAID licoferone, which inhibits both COX and 5-lipoxygenase (5-LOX), has also been shown to decrease CRC membrane fluidity leading to the inhibition of EGFR signaling, which contributes to apoptosis [62]. Thus, NSAIDs could be another attractive agent to target EGFR by co-treatment with other EGFR target drugs.

3.2. E-cadherin

E-cadherin is a calcium-dependent type-1 transmembrane protein. It is important for tight cell-cell junctions and anchors to the cytoskeleton. E-cadherin can play an important role in tumor progression through its sequestration of β -catenin to the plasma membrane, which

prevents β -catenin from acting in the Wnt signaling pathway and from performing its job as a cell adhesion molecule. Loss of E-cadherin function and/or expression is an important step in epithelial-mesenchymal transition (EMT) leading to invasion and migration. In an animal model, sulindac treatment protected $APC^{Min/+}$ mice from E-cadherin loss and accumulation of nuclear β -catenin in colon tumors [63]. On the contrary, the non-selective NSAID indomethacin reduced protein levels of E-cadherin and collagen IV, while increasing the activity of matrix metalloproteinase-9 (MMP-9) leading to enhanced motility *in vitro* in lung cancer cell lines [64]. Furthermore, low doses of celecoxib treatment resulted in loss of E-cadherin, followed by induction of EMT [65]. Thus, further study may need to clarify the role of E-cadherin in NSAID-induced anti-tumorigenesis.

3.3. Phosphodiesterase-5 (PDE5)

Accumulating evidence suggests that modulating the intracellular secondary messengers, cAMP and cGMP may be a promising approach in the field of anti-cancer therapy. Inhibiting PDE5 leads to increased intracellular cGMP levels and activated cGMP-dependent protein kinase (PKG). PDE5 was found to be overexpressed in colorectal cancer cells as well as in colon cancer tissues compared to adjacent normal tissues [66]. Indeed, Dr. Piazza's group has reported that SS and other NSAIDs inhibit PDE5 activity, and this leads to induction of apoptosis in cancer cells [67]. Therefore, inhibition of PDE5 may contribute to the anti-cancer properties of NSAIDs. Since PDE5 inhibitors have been previously reported to have chemo-sensitizing effects [68], NSAIDs like SS may be useful chemo-sensitizers along with other cancer drugs.

4. Cytoskeletal proteins

Cytoskeletal proteins are critical for maintaining normal cellular form and function. They represent the highways for vesicle trafficking, which is a key element of autocrine and paracrine cellular signaling. Cytoskeletal protein rearrangement is also a critical part of cell migration in cancer. All of these aspects are areas that would need to be dysregulated to allow a pre-cancerous cell to go through EMT and metastasize to new sites.

4.1. Nesprin-2

Nesprin-2 is a 796 kDa protein containing an N-terminal actin-binding domain consisting of two calponin homology domains, a central coiled-coil rod domain, and a C-terminal transmembrane KASH domain responsible for targeting to the nuclear envelope and interaction with inner nuclear membrane SUN proteins [69–71]. Also, the Nesprin-2/SUN complex is important for the proper nuclear positioning that is necessary for fibroblast migration, suggesting that Nesprin-2 may have an important role in cell motility [72]. Recently, a link between Nesprin-2 and cancer has been suggested because it was observed in tumor tissues more frequently and at higher expression levels than normal tissues [73]. SS and other NSAIDs down-regulate Nesprin-2 expression in human colorectal cancer cells and its down-regulation is highly associated with NSAIDs' effect on anti-tumorigenesis as assessed by cell impedance experiments [73]. Therefore, Nesprin-2 is likely a molecular target of NSAIDs in a group of cell assembly proteins.

5. TGF- β signaling

The mixtures of cytokines that are produced in the tumor microenvironment have important roles in cancer. TGF- β is a multifunctional regulatory polypeptide that is part of a large family of cytokines that control many aspects of cellular function, including cellular proliferation, differentiation, migration, apoptosis, adhesion, angiogenesis, immune surveillance, and survival. TGF- β signaling can drive either tumor suppression or tumor

promotion. The TGF- β superfamily protein NAG-1 has been extensively studied in NSAID-induced anti-tumorigenesis.

5.1. NAG-1

NAG-1, also known as GDF15, MIC-1, and PDF, is a divergent member of the TGF- β superfamily. NAG-1 is one of the few TGF- β superfamily proteins whose receptors remain unknown. NAG-1 is highly induced by most conventional NSAIDs [74] and its induction by NSAIDs is mediated in a COX-independent manner [75]. NAG-1 induction by NSAIDs not only plays a role in colorectal anti-tumorigenesis, but also in others including head and neck cancer [76], prostate cancer [77], ovarian cancer [78], and thyroid cancer [79]. Mechanistically, it has been reported that NSAIDs up-regulate EGR-1 *in vitro* and *in vivo*, and HER-1 then binds to the promoter region of NAG-1 [14, 80]. SS also induces NAG-1 expression, and its induction can be blocked by dominant negative PERK and by dominant negative CHOP [28], indicating that NAG-1 is downstream of PERK and CHOP (Fig. 1). Since NAG-1 transgenic mice exhibit anti-cancer activity and anti-inflammation activity [81, 82] in the colon and lung, NAG-1 must be a key target protein to control anti-inflammation and anti-cancer activity, mediated by NSAIDs.

6. Cell cycle regulators

Several studies have shown that NSAIDs can modulate cell cycle progression. Expression and activity of cyclins and CDKs are modulated by several NSAIDs in a COX-independent manner. Two important cell cycle regulators, cyclin D1 and p21 are discussed.

6.1. Cyclin D1

Cyclin D1 serves as a key regulator in cell cycle progression by activating cyclin dependent kinase (CDK) 4/6 and is frequently over-expressed in tumor cells. There is a substantial body of experimental evidence showing that cyclin D1 could be a potential cancer therapeutic target [83]. NSAIDs, as potential anti-cancer agents, have been well documented to down-regulate cyclin D1 accompanied by G1 cell cycle arrest; however, the underlying mechanisms could be diverse. Various NSAIDs can down-regulate cyclin D1 expression by suppressing NF- κ B activation in the tumor necrosis factor (TNF)-induced KMB-5 cell model [84]. Also, aspirin cause cyclin D1 degradation in SW480 cells through the p38 MAP kinase pathway, which, in turn, results in NF- κ B pathway activation and subsequently apoptosis [85]. On the other hand, the suppression of Wnt/ β -catenin signaling by NSAIDs could also contribute to cyclin D1 down-regulation in colorectal cancer cells [86–90]. Although TA-mediated cyclin D1 decreases have been proposed to result from degradation of Sp transcription factor protein [91], we recently showed that TA can inhibit cyclin D1 translation via PERK/eIF2 α pathway activation in colorectal cancer cells [9]. Apparently, NSAIDs alter cyclin D1 expression by various transcriptional and post-transcriptional mechanisms.

6.2. p21

SS increases p21 mRNA expression in the immortalized human breast epithelial cell line MCF-10F [92] and increases p21 protein in ovarian cancer cells [78]. Celecoxib also increases p21 protein expression in the human CRC cell lines HCT-15, Caco-2, and HT-29 [93]. COX-2-preferential NSAIDs NS398 and nimesulide also increase p21 expression at the transcriptional level [94]. Collectively, a decrease in p21 expression may be one of the main oncogenic events in the development of cancer, and thus p21 induction by NSAIDs could be the molecular link between NSAIDs and chemopreventive activity in several cancers.

7. Conclusion

NSAIDs interact with many pathways in cancer cells. There are many promising cyclooxygenase-independent mechanisms utilized by NSAIDs that provide potential avenues for developing new and better drugs that minimize or eliminate the undesirable side effects of cyclooxygenase inhibition such as gastric bleeding and cardiovascular risks. NSAIDs up-regulate a number of transcription factors such as the tumor suppressors EGR-1, ATF3, and CHOP and down-regulate oncogenic transcription factors Sp1 and β -catenin (Fig. 1). The oncogenic cell membrane protein EGFR is down-regulated, and the activity of the metastatic protein complex Smad2/3 is decreased by NSAID treatment in CRC cell lines, while the cell adhesion protein E-cadherin is protected in APC^{Min/+} mice from loss of expression by NSAID treatment (Fig. 2). Some NSAIDs also affect cytoskeletal reorganization and loss of actin stress fibers. The secreted tumor suppressor protein NAG-1 is induced by NSAIDs both *in vitro* and *in vivo* via p53, EGR-1, CHOP, and other pathways. Thus, NSAIDs and improved derivatives of NSAIDs have great potential as both chemotherapeutic and chemopreventive agents in cancer. Molecular targets of two NSAIDs, SS and TA, are summarized in Table 1, and more targets could be identified in the future.

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Abbreviations

AA	arachidonic acid
NSAID	non-steroidal anti-inflammatory drug
FAP	familial adenomatous polyposis
TA	tolfenamic acid
SS	sulindac sulfide
EGR-1	early growth response-1
NAG-1	NSAID-activated gene-1
EMT	epithelial-mesenchymal transition
bZIP	basic leucine zipper domain
EGFR	epidermal growth factor receptor
PDE5	phosphodiesterase type 5
TGF-β	transforming growth factor-beta
CRC	colorectal cancer
CHOP	C/EBP homologous transcription factor
SP	specific protein

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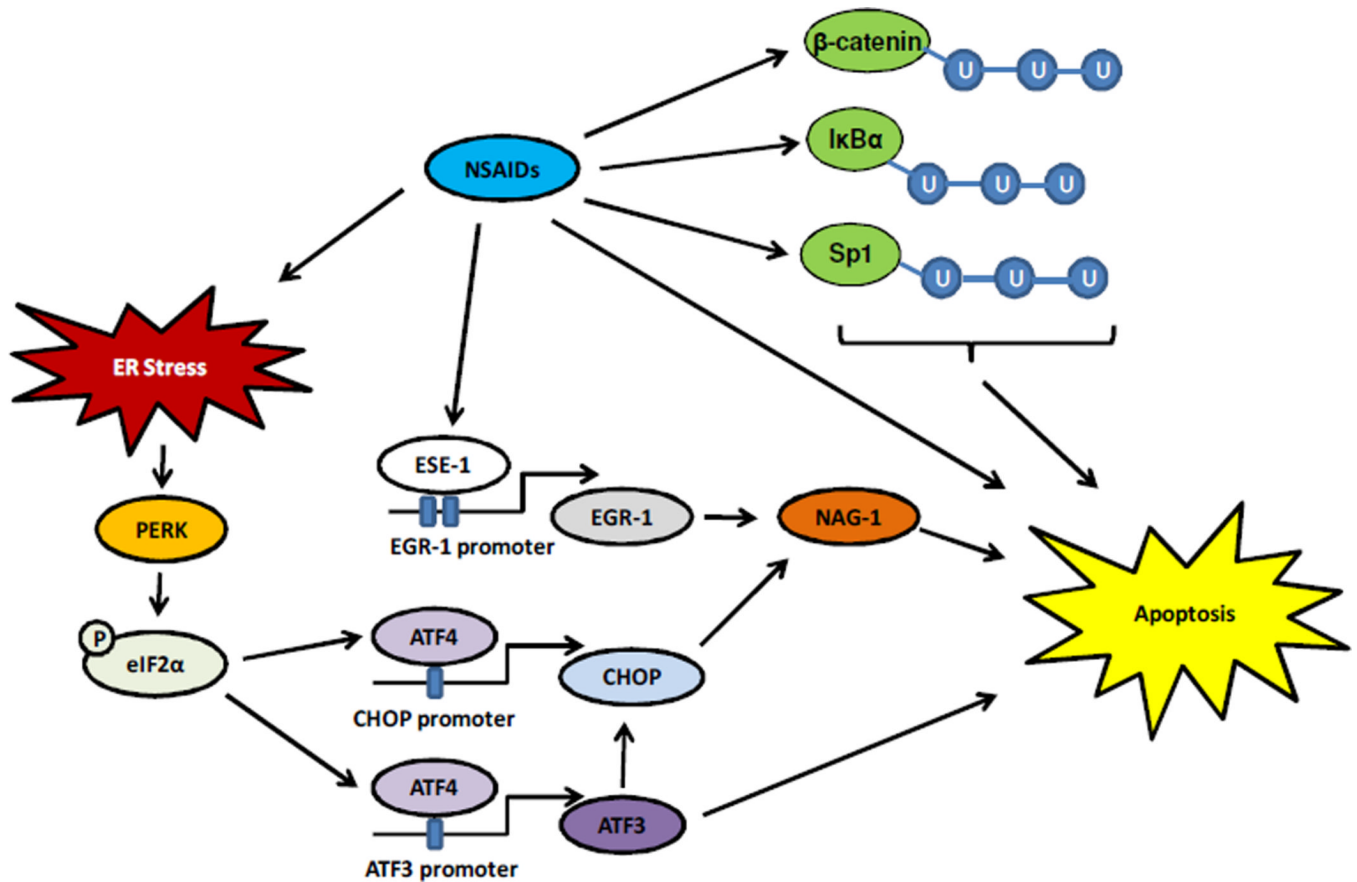


Fig. 1. NSAIDs' effects on transcriptional factors. NSAIDs induce apoptosis through a variety of transcription factors and pathways. Pro-tumorigenic proteins such as β -catenin, I κ B α , and Sp1 are degraded by NSAIDs through ubiquitination pathways. NSAIDs can also induce ESE-1 nuclear translocation, where it binds to EGR-1's promoter and up-regulates pro-apoptotic NAG-1 protein expression. ER stress is induced by NSAID treatment as well, which causes the ER membrane protein PERK to phosphorylate eIF2 α . The phospho-eIF2 α protein activates ATF4, which translocates to the nucleus where it binds to the promoter regions of the transcription factors CHOP or ATF3. This leads to up-regulation of CHOP and NAG-1. NSAIDs also induce apoptosis via unknown mechanisms. All of these pathways funnel into induction of apoptosis.

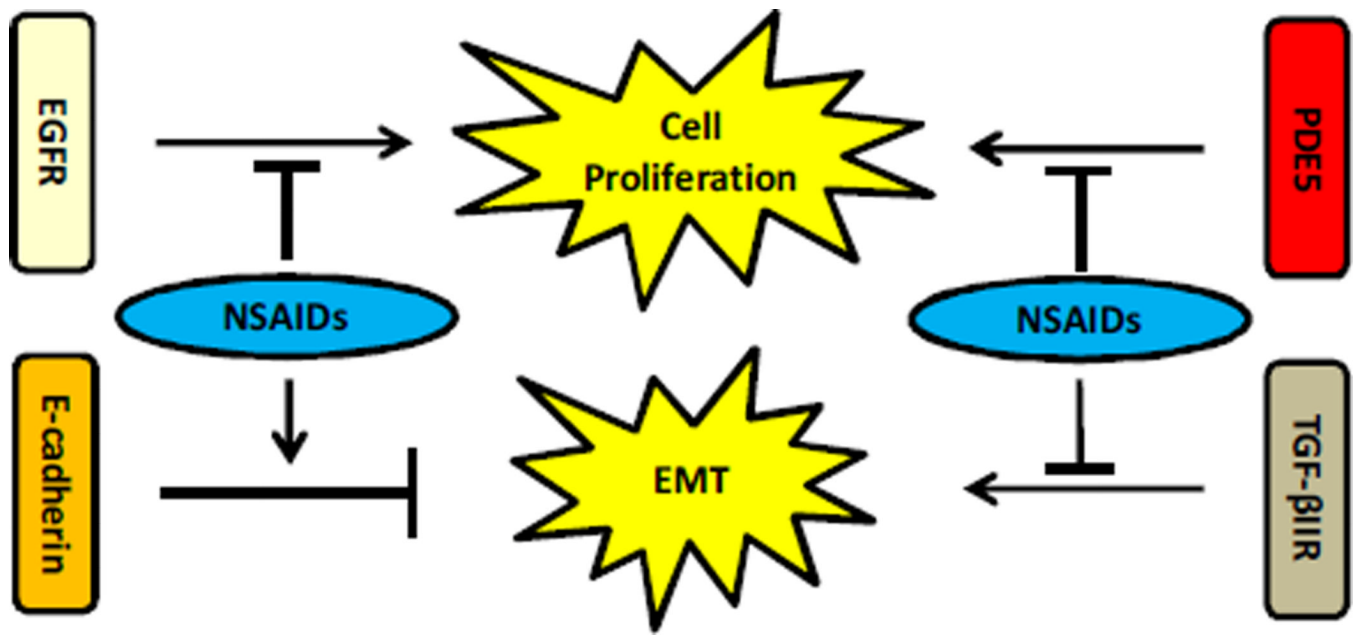


Fig. 2. NSAIDs' effects on cell signaling proteins. EGFR signaling and PDE5's effect on cAMP and cGMP drive increased cell proliferation. NSAIDs inhibit cell proliferation through down-regulation of EGFR protein expression and inhibition of PDE5 activity. E-cadherin is an adhesion molecule that is important for maintaining tight cell junctions and is lost or reduced during EMT. TGF- β signaling also drives EMT. NSAIDs block EMT through reduction of Smad activity or serum levels of TGF- β 1 ligand, and through protection of E-cadherin protein expression.

Table 1

Molecular targets of sulindac sulfide and tolfenamic acid in different cancer types.

NSAIDs	Up-regulated targets	Down-regulated targets	Cancer types [References]
Sulindac sulfide	EGR-1		Colon [80], Lung [13], brain [95]
	ATF3		Colon [20]
		NF-κB	Colon [46], Breast [96]
	NF-κB		Colon [50]
		β-catenin	Colon [53], lung and breast [97]
		EGFR	Colon [61]
	E-cadherin		Colon [63]
		PDE5	Colon [67]
	NAG-1		Colon [74], ovarian [78], Gastric [98], Prostate [99]
		Nesprin-2	Colon [73]
	p21		Breast [92], ovarian [78], Head/Neck [100], Pancreas [101]
	CHOP		Colon [28]
	Cyclin D1	Breast [92], pancreas [101], colon [102]	
Tolfenamic acid	EGR-1		Colon [15]
	ATF3		Colon [19]
	ATF4		Colon [9]
	CHOP		Colon [9]
		Sp1	Pancreas [36], Rhabdomyosarcoma [37], lung [38], esophageal [91]. Ovarian [103], colon [40]
	NF-κB		Colon [49]
		Smad2/3	Lung [60]
	NAG-1		Colon [15], Oral [76], thyroid [79]
	p21		Breast [104]
		Cyclin D1	Colon [9]