A novel plausible mechanism of NSAIDs-induced apoptosis in cancer cells: the implication of proline oxidase and peroxisome proliferator-activated receptor

Adam Kazberuk1 · Ilona Zareba1 · Jerzy Palka1 · Arkadiusz Surazynski1

Received: 1 April 2020 / Revised: 19 May 2020 / Accepted: 14 July 2020 / Published online: 24 July 2020
© The Author(s) 2020

Abstract
Although pharmaco-epidemiological studies provided evidence for the anticancer potential of non-steroidal anti-inflammatory drugs (NSAIDs), the mechanism of their anti-cancer activity is not known. Several lines of evidence suggest that proline dehydrogenase/proline oxidase (PRODH/POX) may represent a target for NSAIDs-dependent anti-cancer activity. PRODH/POX catalyzes conversion of proline into Δ1-pyrroline-5-carboxylate releasing ATP or reactive oxygen species for autophagy/apoptosis. Since NSAIDs are ligands of peroxisome proliferator-activated receptor (PPARs) and PPARs are implicated in PRODH/POX-dependent apoptosis we provided a hypothesis on the mechanism of NSAIDs-induced apoptosis in cancer cells.

Keyword Cancer cells · Apoptosis · Non-steroidal anti-inflammatory drugs · Peroxisome proliferator-activated receptor · Proline dehydrogenase · Proline oxidase

Anticancer activity of NSAIDs
Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of drugs commonly prescribed due to their wide spectrum of pharmacological effects. However they are preferred for the treatment of inflammatory diseases. The molecular mechanism of NSAIDs action is related to the inhibition of cyclooxygenases (COX-1 and COX-2), enzymes catalyzing the biosynthesis of prostaglandins (PGs) from arachidonic and linoleic acids. COX-1 is expressed constitutively in most mammalian cells and maintains homeostasis of some physiological processes, while COX-2 is induced in response to inflammation [1]. While inhibition of COX-1 evokes antiplatelet effect, inhibition of COX-2 has strong anti-inflammatory, antipyretic and analgesic effects [2, 3].

It is well established that inflammatory environment promotes cancer development. The mechanism of this process is due to increased levels of COX-2 and prostaglandin E2 (PGE2) [4–7] that promote proliferation, migration, invasion, and cell adhesion [8, 9]. According to these facts, medication with NSAIDs was associated with decreased risk of certain cancer types, particularly gastrointestinal tract cancers (gastric or colorectal cancer), lung, breast, and prostate cancers [10–14]. Clinical and pharmacoepidemiological studies provide evidence that aspirin and other cyclooxygenase-2 enzyme inhibitors lower recurrence of colorectal cancer by about 20% [12, 15, 16]. Another example is that regular, non-selective COX-2 NSAIDs treatment (i.e. aspirin and ibuprofen) caused a 69% reduction in the relative risk of lung cancer [17]. The explanation for the potential mechanism of anticancer activity of NSAIDs comes from studies on the inhibitory effect on cyclooxygenases that are frequently overexpressed in different types of cancer [18, 19]. Such a mechanism was observed in cultured HT-29 human colon cancer cells where apoptosis occurred after incubation with sulindac and sulindac sulfide, salicylate and other NSAIDs [20]. COX-2 inhibition attenuates also angiogenesis through expression of vascular endothelial growth factor (VEGF) and metalloproteinases [21]. However, some experiments
show that the anti-neoplastic effect of NSAIDs is more complex and cannot be explained on the basis of cyclooxygenase inhibition pathway [22]. In human prostate cancer cell lines, PC3 and LNCaP which are lacking COX-2, the treatment with selective COX-2 inhibitor, celecoxib inhibited the growth of both cell lines independently of PGE\textsubscript{2} level. The similar effect was observed in vivo [23, 24]. Other representative studies carried out using human colon cancer HT-29 cells expressing COX-1 and -2 and HCT-15 lacking both isoforms of cyclooxygenase confirmed prostaglandin-independent effects of NSAIDs. However, the concentrations of NSAIDs required for inhibition of COX and cancer cell proliferation are different [20, 25]. The concentration of NSAIDs required for inhibition of cell proliferation is much higher than those for inhibition of cyclooxgenases activity. Another evidence for COX-independent effect of NSAIDs was provided by studies on chiral centers of ibuprofen and flurbiprofen. When the drugs are \(S\)-enantiomers they evoke non-selective COX inhibition while \(R\)-enantiomers are deprived of both COX-1 or COX-2 inhibitory activity. However, both \(S\)- and \(R\)-enantiomers have the same anti-proliferative effects. It has been suggested that this effects of NSAIDs can be related to inhibition of cyclic guanosine monophosphate phosphodiesterases (cGMP PDEs) signaling, Wnt/\(\beta\)-catenin signaling, peroxisome proliferator-activated receptors, retinoid X receptors, IKK\(\beta\)/NF-\(\kappa\)B, PDK-1/AKT, Akt/mTOR signaling inhibition and AMP-activated protein kinase (AMPK) up-regulation [26–28].

Another possible pathway potentially involved in NSAIDs induced apoptosis in cancer cells is related to the activity of 15-lipoxygenase-1 (15-LOX-1). COX and LOX are the major enzymes responsible for polyunsaturated fatty acids metabolism. In vitro and in vivo studies indicated that gene expression of 15-LOX-1 and level of its main product, 13-hydroxyoctadecadienoic acid (13-S-HODE) is significantly decreased in adenomas or carcinomas comparing to normal mucosa [29, 30]. LOX is the main enzyme metabolizing colonic linoleic acid to eicosanoids. In-vitro experiments with colon cancer cells that have a different level of COXs expression show that NSAIDs (e.g. sulindac sulfone) can up-regulate 15-LOX-1 expression and increase the formation of 13-S-HODE—the main metabolic product of this enzyme. These effects were related to the apoptosis induction in colon cancer cells and LOX-dependent apoptosis was reversed by using caffeic acid—a 15-LOX-1 inhibitor. Interestingly when the cells were incubated with sulindac sulfone, caffeic acid and 13-S-HODE, apoptosis was significantly elevated but the substitution of 13-S-HODE by linoleic acid had no effect in this combination. One explanation of this effect can be a shift of substrate away from the COXs and toward the LOXs [31]. Another possibility could be the interaction between LOX activity and peroxisome proliferator-activated receptors (PPARs). Increased level of 13-S-HODE, in response to 15-LOX-1 activation can be responsible for significant down-regulation of peroxisome proliferator-activated receptor 6 (PPAR\(\delta\)) in RKO and DLD-1 colon cancer cells. Linoleic acid as a substrate for 15-LOX-1 did not have the same effect alone. Further experiments proved that molecular mechanism for this effects is also related to 13-S-HODE direct binding with PPAR\(\delta\) and downregulation of its expression [32, 33] or even direct 15-LOX-1 to tumor suppressor protein (p53) interaction independently of lipoxygenase enzymatic activity [34]. Another important fact is that products of 15-LOX-1 are well-known ligands of peroxisome proliferator-activated receptor \(\gamma\) (PPAR\(\gamma\)). In vitro model with macrophages or HCT-116 and KO1 cells proved that products of lipid oxidation, particularly 13-S-HODE are effective PPAR\(\gamma\) activators and can promote apoptosis. Some studies have revealed that native LDL had no influence on reporter PPAR\(\gamma\) activity even when high concentrations were used. When PPAR\(\gamma\) reporter activity was stimulated at low concentration of 13-S-HODE [35] it contributed to 70% PPAR\(\delta\) downregulation [32]. Therefore, it has been suggested that NSAIDs-dependent pro-apoptotic activity is mediated by PPARs. Although native LDL has no influence on PPAR\(\gamma\) activation, such an activity evokes oxidized LDL (oxLDL) that mediates PPAR\(\gamma\) transcription [35].

All those signaling pathways are linked to PPAR\(\gamma\)-dependent functions. NSAIDs-dependent activation of PPAR\(\gamma\) down-regulate pro-survival pathways (e.g. Wnt/\(\beta\)-catenin and Akt/mTOR signaling) and up-regulate pro-apoptotic signaling (e.g. AMPK or LOX-1 activated PPAR\(\gamma\) and PRODH/POX).

**Molecular polymorphism and function of PPAR**

The peroxisome proliferator-activated receptors (PPAR) are ligand-dependent transcription factors belonging to the nuclear hormone receptor family. PPARs can regulate the transcription of multiple genes in response to activation by natural or synthetic ligands. Although PPARs regulate lipid metabolism, glucose homeostasis, and adipogenesis [36], they can also affect inflammation, proliferation, differentiation, and carcinogenesis [37, 38]. Activation of PPAR requires heterodimerization with retinoid X receptors (RXR) to form PPAR/RXR complex which binds to specific DNA fragment called PPAR response element (PPRE) in a target gene [39]. Three different isoforms of PPAR are known: PPAR\(\alpha\) (NR1C1), PPAR\(\beta/\delta\) (NR1C2) and PPAR\(\gamma\) (NR1C3) and they all are encoded by different genes [40–42]. Isoforms \(\alpha\), \(\beta/\delta\), \(\gamma\) are differentially expressed in embryonic and adult tissues [43]. PPAR\(\alpha\), PPAR\(\beta/\delta\) and PPAR\(\gamma\) have similar structural and functional domains specific for this
group of the nuclear receptors. Four of them: A/B, C, D, and E/F domains have been determined [44]. N-terminal A/B ligand-independent transactivation function domain (activation function—AF-1) and its activity is related to phosphorylation or sumoylation. Depending on receptor isotype it can be active or non-active in the basal state. DNA-binding domain C contains two highly conserved zinc finger motifs which bind hormone response element (HRE) and recognizes the promoter region of target genes known as PPRE. Flexible hinge region with activity of docking site for cofactors is in D domain and E/F domain with ligand binding domain (LBD) which is C-terminal plays important role in activation of PPAR and PPRE interaction. This results in amplifying of target genes expression with important role of activation function-2 (AF-2) [45, 46]. If the ligand is not present, PPARs can heterodimerize with RXR and bind to promoter region of target gene and recruit corepressors like N-CoR or SMRT resulting in inhibition of gene expression. Conformational changes in PPAR structures (due to ligand binding), lead to corepressor dissociation and interaction with coactivator initiating gene expression [47].

PPARα was the first cloned isoform of this receptor family [48]. It regulates the genes expression involved in cholesterol transport and free fatty acids (FFAs) metabolism through the β-oxidation and peroxisomal pathways [49, 50]. PPARα serves as the main regulator of lipid metabolism in the liver [51]. It is highly expressed in tissues catalyzing fatty acids such as skeletal muscle, brown adipose, kidney, heart, and liver [52, 53]. Moreover these receptors are expressed in vascular and immune cells [54] and also in the hippocampus and hippocampal neurons [55]. Activation of PPARα by statins like simvastatin leads to increased neurotrophins expression which is important in processes of learning and memory [56].

PPARβ/δ have ubiquitous localization with high expression in the intestine, liver, abdominal adipose tissue, skeletal muscle, liver. They regulate glucose and cholesterol level in blood and are involved in lipid metabolism [57, 58]. PPARβ/δ function as a transcriptional repressor in its unliganded state what differs them from PPARα and PPARγ. Repression of basal transcription as well as PPARα- and PPARγ-mediated transcription can occur due to unliganded PPARβ/δ through the corepressor recruitment. PPARβ/δ can inhibit PPARα and PPARγ activity by isotype-specific repression due to PPRE sites competition [59]. Activation of PPARβ/δ has also pro-tumorigenic effect in breast cancers. Fatty acid-binding protein 5 (FABPS) interaction with PPARβ/δ mediates epidermal growth factor receptor (EGFR) dependent cell proliferation. GW501516, GW0742, and L-165041 are the synthetic ligands with very high affinity to β/δ isoform at low concentrations (1.1 nM for GW501516, 1.0 nM for GW0742 and 50 nM for L165041) and significant selectivity over the other isoforms of PPARs [60].

Selective PPARβ/δ agonist GW501516 accelerated tumor formation in mice while inverse agonist inhibited PPARβ/δ targeting genes related to MDA-MB-231 cell invasion. High expression of PPARβ/δ in MCF-7 enhanced cell migration and increased resistance to endoplasmic reticulum stress conditions as low glucose and hypoxia. This suggests their important role in the adaptation of breast cancer cells to different micro-environmental stress conditions [61]. It was found that overexpression of PPARβ/δ in human cancers promotes tumor growth by increasing VEGF expression and activating PI3K-Akt signaling supporting cell survival [62]. Moreover, higher expression of PPARδ in chronic lymphocytic leukemia (CLL) and other hematologic cancers was found to support growth in stress conditions as hypoxia, low glucose, and exposure to cytotoxic drugs. Synthetic PPARδ antagonists and genetic deletion of PPARδ reversed its growth supporting activity [63].

PPARγ is widely expressed in brown and white adipose tissue, spleen and large intestine [64, 65]. PPARγ has three isoforms which are transcribed on the same gene but undergo control of different promoters [66]. γ1 and γ3 have different mRNA but the protein is the same for both isoforms [64]. γ1 is present in brown and white adipose tissue, large intestine, immune cells, pancreas, liver, small intestine and kidney [67]. Low level of expression is in central nervous systems like in astrocytes, neurons, microglia, and oligodendrocytes. Isoform γ2 is present only in adipose tissue and differs from others due to an additional 30 amino acids on the N-terminal site [68, 69]. Targeting PPARγ was used for the type 2 diabetes treatment with thiazolidinedione (TZD) class of drugs i.e. rosiglitazone, troglitazone, and pioglitazone [70]. PPARγ agonists have been also shown to function as an anticancer factors, especially for obesity related cancers as a prostate, breast, colon, liver, thyroid, lung, and pituitary cancers [71]. It has been linked to the anti-inflammatory activity of PPARs. Anti-inflammatory effects of PPARγ were observed due to inhibition of tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), interleukin 6 (IL-6) and PGE2 production [72]. PPARγ is expressed in breast adenocarcinoma, human liposarcoma and some of colonic cancer cell lines [73]. Activation of PPARγ contributes to lowering the level of angiogenic factors and reduction in migration and proliferation of endothelial cells [74]. Ligand activation of PPARγ by troglitazone promotes TRAIL-induced apoptosis in human lung cancer via autophagy [75]. Adenovirus gene transferred SNU-668 gastric cancer cells with overexpression of PPARγ presented significant the growth inhibition and apoptosis activation due to strong IGFBP-3 upregulation. Insulin-like growth factor-binding protein-3 IGFBP-3 is a tumor suppressor gene, independent of IGF signaling [76]. Some reports proved that activation of PPARγ can inhibit the growth of ovarian cancer by suppressing proto-oncogene B-cell lymphoma 3-encoded protein (BCL3) in
response to microRNA-125b (miR-125b) tumor suppressor upregulation. In fact in bladder, breast, ovarian and lung cancer, it was observed that miR-125b tumor suppressor level was downregulated and this affected IGF, PI3K/Akt/mTOR, and mitogen activated protein kinase (MAPK) signaling pathways [77]. Although the PPARγ activation contributes to the pro-apoptotic phenotype of cancer cells, the molecular mechanism of this process is still unknown. One of the enzymes regulated by PPARγ and involved in cell death is proline oxidase (POX).

**PRODH/POX-dependent apoptosis**

Proline oxidase (POX) also known as a proline dehydrogenase (PRODH) is an inner mitochondrial membrane flavin-dependent enzyme which catalyzes the conversion of l-proline to Δ1-pyrroline-5-carboxylate (P5C). This process donates electrons through flavin adenine dinucleotide (FAD) to the electron transport chain for ATP generation. From this point of view the activity of PRODH/POX promotes transporters are directly transferred to oxygen forming superoxide radicals and other reactive oxygen species (ROS) leading to this point of view the activity of PRODH/POX promotes proto-oncogene c-myc (MYC) expression. However, depending on environmental conditions superoxide radicals generated by PRODH/POX-dependent functions [83].

The important role of tumor microenvironment in PRODH/POX-dependent apoptosis is also derived from PRODH/POX-dependent apoptosis due, to caspase 9 activation, cytochrome c release and nuclear condensation/fragmentation independently of p53 contribution [80]. Studies of recent years have established an important role of proline in cancer cell metabolism. Understanding the role of this amino acid in regulation on cell survival and death focused therefore on enzymes involved in proline cycling. PRODH/POX and pyrroline-5-carboxylate reductase (P5CR, a.k.a. PYCR) are of special interest. Ornithine or glutamate are substrates for proline synthesis and both of them leads to l-glutamate-γ-semialdehyde (GSAL) production, which can be converted reversibly and spontaneously into P5C. Transformation of ornithine to GSAL is possible due to ornithine δ-aminoacidotransferase (OAT), while P5C synthase (P5CS) catalyzes the process of P5C synthesis from l-glutamate with GSAL intermediate. These steps of proline biosynthesis occur in mitochondria. If P5C is transferred to the cytosol, then it can be reduced to proline. This process is catalyzed by P5C reductase (PYCRL) which is the NADPH-dependent enzyme. A similar reaction can take place in mitochondria, but in this case different isoform of this enzyme as PYCR1 or PYCR2 are involved. It was proved that knockdown of PYCR1 can be also responsible for reduced cell proliferation in liver cancer [87]. Proline metabolite—glutamate—glutamate by further conversion to αKG by glutamate dehydrogenase can enter the TCA cycle and contribute to cellular energy production. Another issue is the role of proline cycle in cell proliferation and biomass production through the link to the pentose phosphate pathway. During this step some precursors of nucleotides required for DNA and RNA synthesis are produced [88, 89]. However proline for PRODH/POX-dependent apoptosis is also derived from collagen degradation products. Proline and hydroxyproline constitute about 25% of residues in collagen [90]. The most important process supporting intracellular proline is regulated by prolidase.
The role of prolidase in PRODH/POX-dependent apoptosis

Prolidase (PEPD, peptidase D or iminopeptidase) is imidodipeptidase or imido-tripeptidase localized in the cytoplasm [91, 92] and its function is to cleave imido-peptides with C-terminal proline or hydroxyproline [93]. They are derived mainly from collagen degradation products [94, 95]. In the α1 subunit of type I procollagen, proline forms 119 bonds with glycine and in α2 subunit such a doublet occurs 106 times. Although in matured collagen proline is mostly hydroxylated. Un-hydroxylated proline in glycine–proline (gly–pro) doublet occurs 25 times [95]. Therefore collagen degradation significantly contributes to intracellular proline concentration. It is known that prolidase activity is an important factor for proline recycling for collagen re-synthesis and therefore the enzyme plays a step limiting role in the regulation of collagen biosynthesis. The importance of this iminopeptidase in regulation of collagen biosynthesis was documented in fibroblast treated with proline metabolite—P5C [96], anti-inflammatory drugs [97], during experimental fibroblasts aging [98], experimental chondrocytes inflammation [99], activation of integrin receptor for type I collagen [100], in fibroblast-derived from osteogenesis imperfecta affected patients [101] and in several cancer tissues [102–104]. It was also found that prolidase may act at the level of transcription factors regulation. In colorectal cancer cells prolidase overexpression was correlated with increased levels of nuclear hypoxia inducible factor 1α (HIF-1α) and HIF-1α-dependent gene products like a vascular endothelial growth factor (VEGF) and glucose transporter-1 (Glut-1)—important factors in cancer progression [105]. Suppressed proteasomal degradation of HIF-1α and increased HIF-1α transcriptional activity occurs when HIF prolyl hydroxylase activity is inhibited by proline. The increased HIF-1α transcriptional activity is due to increased concentration of cytoplasmic proline, as a result of prolidase overexpression. Activation of HIF-1α related pro-survival signaling pathways undergoes through inflammatory and pro-angiogenic genes (eg. COX-2, TNFα, IL-1, NFκB, VEGF) [106]. It suggests that prolidase activity plays important role in regulation of HIF-1α-dependent functions.

All these data suggest COX-independent mechanisms of NSAID-dependent apoptosis in cancer cells. Until now numerous investigations were conducted to confirm that cancer cells treatment with NSAIDs are associated with downregulation of oncogenic factors expression and up-regulation of apoptosis pathway with significant role of the PPARγ [12–16]. Since NSAIDs are ligands of PPARγ and PPARγ induces PRODH/POX-dependent apoptosis, this sequence of events may represent the mechanism of anticancer activity of NSAIDs.

Conclusions

Studies of last decade provided evidence for the role of PRODH/POX and PPARs in the regulation of apoptosis/autophagy in cancer cells. PRODH/POX expression is often down-regulated in various tumors, limiting mitochondrial proline degradation and PRODH/POX-dependent apoptosis. NSAIDs were shown to stimulate the transcriptional activity of PPARα/γ that are well-characterized PRODH/POX inducers. However, the critical factor for the PRODH/POX-induced apoptosis is proline availability that depends on the activity of prolidase (enzyme supporting cytoplasmic proline level) and the intensity of collagen biosynthesis (proline utilizing process). Although specific environmental conditions may affect PPARs and PRODH/POX it seems that NSAIDs activate PRODH/POX-dependent apoptosis through PPARα/γ. The hypothesis is outlined in Fig. 1.

NSAIDs up-regulate PPARα/γ directly or indirectly (by 15-LOX-1-dependent generation of 13-S-HODE). Up-regulated PPARγ induces transcription of PRODH/POX and subsequently conversion of proline into P5C, generating ROS-inducing apoptosis. The process requires proline availability that is dependent on the activity of prolidase (proline supporting enzyme) and collagen biosynthesis (proline utilizing process). The role of other outlined NSAIDs-dependent pathways in the PRODH/POX-dependent apoptosis are unknown.
**Author contributions** AK: was responsible for preparation of manuscript, data collection and design of the hypothesis; IZ: participated in drafting the article; JP: revised manuscript critically for important intellectual content and provided overview of subject; AS: was responsible for final approval of the version to be published.

**Funding** This research was supported by the National Science Centre, Grant number 2017/27/N/NZ7/02370.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

**References**

1. Usman MW, Luo F, Cheng H, Zhao JJ, Liu P. Chemopreventive effects of aspirin at a glance. Biochim Biophys Acta. 2015;1855:254–63.

2. Marsico F, Puillillo S, Filardi PP. NSAIDs and cardiovascular risk. J Cardiovasc Med (Hagerstown). 2017;18 Suppl 1: Special Issue on The State of the Art for the Practicing Cardiologist: The 2016 Conoscere E Curare II Cuore (CCC) Proceedings from the CLI Foundation: e40–e43.

3. Echizen K, Hirose O, Maeda Y, Oshima M. Inflammation in gastric cancer: interplay of the COX-2/prostaglandin E2 and toll-like receptor/MyD88 pathways. Cancer Sci. 2016;107:391–7.

4. Kim HS, Moon HG, Han W, Yom CK, Kim WH, Kim JH, et al. COX2 overexpression is a prognostic marker for Stage III breast cancer. Breast Cancer Res Treat. 2012;132:51–9.

5. Eibl G, Bruemmer D, Okada Y, Duffy JP, Law RE, Reber HA, et al. PGE(2) is generated by specific COX-2 activity and increases VEGF production in COX-2-expressing human pancreatic cancer cells. Biochem Biophys Res Commun. 2003;306:887–97.

6. Zappavigna S, Cossu AM, Grimaldi A, Bocchetti M, Ferraro GA, Nicoletti GF, et al. Anti-inflammatory drugs as anticancer agents. Int J Mol Sci. 2020;21:2605–34.

7. Hashemi Goradel N, Najafi M, Salehi E, Farhood B, Mortezaee K. Cyclooxygenase-2 in cancer: a review. J Cell Physiol. 2019;243:5683–99.

8. Pang LY, Hurst EA, Argyle DJ. Cyclooxygenase-2 in cancer: a review. J Cell Physiol. 2019;234:5683–99.

9. G. D, Menter. Prostaglandins in Cancer Cell Adhesion, Migration, and Invasion. In: DuBois RN, editor. International Journal of Cell Biology: International Journal of Cell Biology; 2012. p. 1–21.

10. Chan AT, Giovannucci EL, Meyerhardt JA, Schernhammer ES, Curhan GC, Fuchs CS. Long-term use of aspirin and nonsteroidal anti-inflammatory drugs and risk of colorectal cancer. JAMA. 2005;294:914–23.

11. Shebl FM, Hsing AW, Park Y, Hollenbeck AR, Chu LW, Meyer TE, et al. Non-steroidal anti-inflammatory drugs use is associated
with reduced risk of inflammation-associated cancers: NIH-AARP study. PLoS ONE. 2014;9:e114633.
12. Rothwell PM, Wilson M, Elwin CE, Norrving B, Algra A, War- 
low CP, et al. Long-term effect of aspirin on colorectal cancer 
incidence and mortality: 20-year follow-up of five randomised 
trials. Lancet. 2010;376:1741–50.
13. Rothwell PM, Price JF, Fowkes FG, Zanchetti A, Roncaglioni 
MC, Tognoni G, et al. Short-term effects of daily aspirin on 
cancer incidence, mortality, and non-vascular death: analysis of 
the time course of risks and benefits in 51 randomised con-
trolled trials. Lancet. 2012;379:1602–12.
14. Seetha A, Devaraj H, Sudhandiran G, Indomethacin and 
juglone inhibit inflammatory molecules to induce apoptosis in 
colon cancer cells. J Biochem Mol Toxicol. 2020;34:e22433.
15. Cheung KS, Chen L, Chan EW, Seto WK, Wong ICK, Leung 
WK. Nonsteroidal anti-inflammatory drugs but not aspirin are 
associated with a lower risk of post-colonoscopy colorectal 
cancer. Aliment Pharmacol Ther. 2020;51:899–908.
16. Arisan ED, Ergül Z, Bozdağ G, Rencüzoğulları Ö, Çoker-Gür-
kan A, Obakan-Yerlikaya P, et al. Diclofenac induced apoptosis 
via altering PI3K/Akt/MAPK signaling axis in HCT 116 more 
efficiently compared to SW480 colon cancer cells. Mol Biol 
Rep. 2018;45:2175–84.
17. Harris RE, Beebe-Donk J, Schuller HM. Chemoprevention of 
lung cancer by non-steroidal anti-inflammatory drugs among 
cigarette smokers. Oncol Rep. 2002;9:693–5.
18. Chen H, Cai W, Chu ESH, Tang J, Wong CC, Wong SH, et al. 
Hepatic cyclooxygenase-2 overexpression induced spontaneous 
hepatocellular carcinoma formation in mice. Oncogene. 2017;36:4415–26.
19. Majumder M, Xin X, Liu L, Kutous MI, Qiao L, Staiano-Coico 
BK, Celińska-Janowicz K, Miltyk W. Celecoxib in cancer therapy 
and prevention—review. Curr Drug Targets. 2019;20:302–15.
20. Hanif R, Pittas A, Feng Y, Koutsos MI, Qiao L, Staiano-Coico 
et al. Decreased 13-
-15-lipoxygenase-1 is reversed by celecoxib in colorectal cancer. 
Ann Surg. 2005;241:941–6.
21. Shureiqi I, Wojno KJ, Poore JA, Reddy RG, Moussalli MJ, Spin-
dler SA, et al. Decreased 13-5-hydroxyoctadecadienoic acid le-
vels and 15-lipoxygenase-1 expression in human colon cancers. 
Carcinogenesis. 1999;20:1985–95.
22. Shureiqi I, Chen D, Lotan R, Yang P, Newman RA, Fischer SM, 
et al. 15-Lipoxygenase-1 mediates nonsteroidal anti-inflammato-
ry drug-induced apoptosis independently of cyclooxygenase-2 in 
colon cancer cells. Cancer Res. 2000;60:6846–50.
23. Shureiqi I, Jiang W, Zuo X, Wu Y, Stimmel JB, Leesnitzer LM, 
et al. The 15-lipoxygenase-1 product 13-5-hydroxyoctadecadieno-
ic acid down-regulates PPAR-delta to induce apoptosis in colo-
rectal cancer cells. Proc Natl Acad Sci USA. 2003;100:9968–73.
24. Il Lee S, Zuo X, Shureiqi I. 15-Lipoxygenase-1 as a tumor sup-
pressor gene in colon cancer: is the verdict in? Cancer Metastasis 
Rev. 2011;30:481–91.
25. Zhu H, Glasgow W, George MD, Chryssovergis K, Olden K, 
Roberts JD, et al. 15-Lipoxygenase-1 activates tumor sup-
pressor p53 independent of enzymatic activity. Int J Cancer. 
2008;123:2741–9.
26. Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Oxidized 
LDL regulates macrophage gene expression through ligand activa-
tion of PPARgamma. Cell. 1998;93:229–40.
27. Okazaki S, Shioi R, Noguchi-Yachide T, Ishikawa M, Mak-
ishima M, Hashimoto Y, et al. Structure–activity relationship 
studies of non-carboxylic acid peroxisome proliferator-activat-
ed receptor α/β (PPARα/δ) dual agonists. Bioorg Med Chem. 
2016;24:5455–61.
28. Peters JM, Shah YM, Gonzalez FJ. The role of peroxisome pro-
liferator-activated receptors in carcinogenesis and chemopreven-
tion. Nat Rev Cancer. 2012;12:181–95.
29. Hueodbler D, Rechenmacher M, Lüke F, Vogelhuber M, Puk-
rop T, Herr W, et al. Peroxisome proliferator-activated receptors 
(PPAR)γ agonists as master modulators of tumor tissue. Int J Mol 
Sci. 2018;19:3540–64.
30. Blitek A, Szynmanska M. Peroxisome proliferator-activated recep-
tor (PPAR) isoforms are differentially expressed in peri-implan-
tation porcine conceptuses. Theriogenology. 2017;101:53–61.
31. Sutinen J. The effects of thiazolidinediones on metabolic com-
plications and lipodystrophy in HIV-infected patients. PPAR 
Res. 2009;2009:37524.
32. Kuenzli S, Saurat JH. Peroxisome proliferator-activated recep-
tors in cutaneous biology. Br J Dermatol. 2003;149:229–36.
33. Shiotia M, Fujimoto N, Kashiwagi E, Eto M. The role of nuclear 
receptors in prostate cancer. Cells. 2019;8:602–21.
34. Zhao S, Kanno Y, Li W, Wakatabi H, Sasaki T, Koike K, et al. 
Picrasidine N is a subtype-selective PPARβ/δ agonist. J Nat 
Prod. 2016;79:879–85.
35. Mazzar D, Dhillon R, Waxman J. COX and cancer. QJM. 
2005;98:711–8.
36. Mats P, Jordan P. Beyond COX-inhibition: ‘side-effects’ of ibu-
profen on neoplastic development and progression. Curr Pharm 
Des. 2015;21:2978–92.
37. Patel MI, Subbaramaiah K, Du B, Chang M, Yang P, Newman 
RA, et al. Celecoxib inhibits prostate cancer growth: evidence of 
a cyclooxygenase-2-independent mechanism. Clin Cancer Res. 
2005;11:1999–2007.
38. Tołoczko-Iwanuik N, Dziemiańczyk-Pakiela D, Nowaszewska 
BK, Celniańska-Janowicz K, Miltý W. Celecoxib in cancer therapy 
and prevention—review. Curr Drug Targets. 2019;20:302–15.
39. Rigas B, Shiff SJ. Is inhibition of cyclooxygenase required for 
the chemopreventive effect of NSAIDs in colon cancer? A 
model reconciling the current contradiction. Med Hypotheses. 
2000;54:210–5.
40. Gurpinar E, Grizzle WE, Piazza GA. NSAIDs inhibit tumorigen-
esis, but how? Clin Cancer Res. 2014;20:1104–13.
41. Gurpinar E, Grizzle WE, Piazza GA. COX-independent mecha-
nisms of cancer chemoprevention by anti-inflammatory drugs. 
Front Oncol. 2013:3:181.
42. Akrami H, Moradi B, Borzabadi Farahani D, Mehdizadeh K. 
Ibuprofen reduces cell proliferation through inhibiting Wnt/β 
catenin signaling pathway in gastric cancer stem cells. Cell Biol 
Int. 2018;42:949–58.
43. Heslin MJ, Hawkins A, Boedefeld W, Arnoletti JP, Frolow 
A, Soong R, et al. Tumor-associated down-regulation of 
15-lipoxygenase-1 is reversed by celecoxib in colorectal cancer. 
Ann Surg. 2005;241:941–6.
A novel plausible mechanism of NSAIDs-induced apoptosis in cancer cells: the implication of...

48. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature. 1990;347:645–50.

49. Gampe RT, Montana VG, Lambert MH, Miller AB, Bledsoe RK, Milburn MV, et al. Asymmetry in the PPARgamma/RXRa ha crystal structure reveals the molecular basis of heterodimerization among nuclear receptors. Mol Cell. 2000;5:545–55.

50. Tenenbaum A, Motro M, Fisman EZ. Dual and pan-peroxisome proliferator-activated receptors (PPAR) co-agonism: the bezafibrate strains. Cardiovasc Diabetol. 2005;4:14.

51. Kersten S, Stienstra R. The role and regulation of the peroxisome proliferator activated receptor alpha in human liver. Biochimie. 2017;136:75–84.

52. Neschen S, Morino K, Dong J, Wang-Fischer Y, Cline GW, Romanelli AJ, et al. n-3 Fatty acids preserve insulin sensitivity in vivo in a peroxisome proliferator-activated receptor-alpha-dependent manner. Diabetes. 2007;56:1034–41.

53. Lo Verme J, Fu J, Astarita G, La Rana G, Russo R, Calignano A, et al. The nuclear receptor peroxisome proliferator-activated receptor-alpha mediates the anti-inflammatory actions of palmitoylethanolamide. Mol Pharmacol. 2005;67:15–9.

54. Yoon M. PPARα in Obesity: Sex Difference and Estrogen Involvement. PPAR Res. 2010;2010:584296. https://doi.org/10.1155/2010/584296.

55. Corbett GT, Gonzalez FJ, Pahan K. Activation of peroxisome proliferator-activated receptor α stimulates ADAM10-mediated proteolysis of APP. Proc Natl Acad Sci USA. 2015;112:8445–50.

56. Roy A, Jana M, Kundu M, Corbett GT, Rangaswamy SB, Mishra RK, et al. HMG-CoA reductase inhibitors bind to PPARα to upregulate neurotrophin expression in the brain and improve memory in mice. Cell Metab. 2015;22:253–65.

57. Grygel-Görniak B. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications—a review. Nutr J. 2014;13:17.

58. Liu Y, Colby JK, Zuo X, Jaoude J, Wei D, Shureiqi I. The role of PPAR-δ in metabolism, inflammation, and cancer: many characters of a critical transcription factor. Int J Mol Sci. 2018;19:3390–404.

59. Tan NS, Vázquez-Carrera M, Montagner A, Song MK, Guillou H, Wahli W. Transcriptional control of physiological and pathological processes by the nuclear receptor PPARγ/δ. Prog Lipid Res. 2016;64:98–122.

60. Palomer X, Barroso E, Zarei M, Botteri G, Vázquez-Carrera M. PPARγ/δ and lipid metabolism in the heart. Biochim Biophys Acta. 2016;1861:1569–78.

61. Müller R. PPARδ in human cancer. Biochimie. 2017;136:90–9.

62. Wang D, Wang H, Guo Y, Ning W, Katkuri S, Wahli W, et al. Crosstalk between peroxisome proliferator-activated receptor delta and VEGF stimulates cancer progression. Proc Natl Acad Sci USA. 2006;103:19069–74.

63. Li YJ, Sun L, Shi Y, Wang G, Wang X, Dunn SE, et al. PPAR-delta promotes survival of chronic lymphocytic leukemia cells in energetically unfavorable conditions. Leukemia. 2017;31:1905–14.

64. Janani C, Ranjitha Kumari BD. PPAR gamma gene—a review. Diabetes Metab Syndr. 2015;9:46–50.

65. Dhaini HR, Dahez Z. Genetic polymorphisms of PPAR genes and human cancers: evidence for gene–environment interactions. J Environ Sci Health Part C. 2019;37:146–79.

66. Fajas L, Aboeuf D, Raspé E, Schoonjans K, Lefebvre AM, Saladin R, et al. The organization, promoter analysis, and expression of the human PPARgamma gene. J Biol Chem. 1997;272:18779–89.

67. Marion-Letellier R, Savoye G, Ghosh S. Fatty acids, eicosanoids and PPAR gamma. Eur J Pharmacol. 2016;785:44–9.

68. Li D, Zhang F, Zhang X, Xue C, Namwanje M, Fan L, et al. Distinct functions of PPARγ isoforms in regulating adipocyte plasticity. Biochem Biophys Res Commun. 2016;481:132–8.

69. Gupta G, Singhvi G, Chellappan DK, Sharma S, Mishra A, Dahiya R, et al. Peroxisome proliferator-activated receptor gamma: promising target in glioblastoma. Panminerva Med. 2018;60:109–16.

70. Fumery M, Speca S, Langlois A, Davila AM, Dubuquoy C, Grauso M, et al. Peroxisome proliferator-activated receptor gamma (PPARγ) regulates lactase expression and activity in the gut. EMBO Mol Med. 2017;9:1471–81.

71. Wang Q, Imam MU, Yida Z, Wang F. Peroxisome proliferator-activated receptor gamma (PPARγ) as a target for concurrent management of diabetes and obesity-related cancer. Curr Pharm Des. 2017;23:3677–88.

72. Han Q, Yuan Q, Meng X, Hua J, Bao Y, Xie G. 6-Shogao attenuates LPS-induced inflammation in BV2 microglia cells by activating PPAR-γ. Oncotarget. 2017;8:42001–6.

73. Kitamura S, Miyazaki Y, Shinomura Y, Kondo S, Kanayama S, Matsuzawa Y. Peroxisome proliferator-activated receptor gamma induces growth arrest and differentiation markers of human colon cancer cells. Jpn J Cancer Res. 1999;90:75–80.

74. Kotlinowski J, Jozkowicz A. PPAR gamma and angiogenesis: endothelial cells perspective. J Diabetes Res. 2016;2016:849235.

75. Nazim UM, Moon JH, Lee YJ, Seol JW, Park SY. PPARγ activation by troglitazone enhances human lung cancer cells to TRAIL-induced apoptosis via autophagy flux. Oncotarget. 2017;8:26819–31.

76. Kim SY, Kim MS, Lee MK, Kim JS, Yi HK, Nam SY, et al. PPARγ induces growth inhibition and apoptosis through upregulation of insulin-like growth factor-binding protein-3 in gastric cancer cells. Braz J Med Biol Res. 2015;48:226–33.

77. Luo S, Wang J, Ma Y, Yao Z, Pan H. PPARγ inhibits ovarian cancer cells proliferation through upregulation of miR-125b. Biochim Biophys Res Commun. 2015;462:85–90.

78. Kononczuk J, Czyzewska U, Moczydlowska J, Surazyński A, Palka J, Miltyk W. Proline oxidase (POX) as a target for cancer therapy. Curr Drug Targets. 2015;16:1464–9.

79. Phang JM. Proline metabolism in cell regulation and cancer biology: recent advances and hypotheses. Antioxid Redox Signal. 2019;30:635–49.

80. Hu CA, Donald SP, Yu J, Lin WW, Liu Z, Steel G, et al. Overexpression of proline oxidase induces proline-dependent and mitochondria-mediated apoptosis. Mol Cell Biochem. 2007;295:85–92.

81. Cappelletti P, Tallarita E, Rabattoni V, Campomenosi P, Sacchi D, Steinfeldt LA, et al. Peroxisome proliferator-activated receptor gamma: promising target in glioblastoma. Panminerva Med. 2015;64:98–122.

82. Liu W, Glunde K, Bhuivalla ZM, Raman V, Sharma A, Phang JM. Proline oxidase promotes tumor cell survival in hypoxic tumor microenvironments. Cancer Res. 2012;72:3677–86.

83. Liu W, Phang JM. Proline dehydrogenase (oxidase), a mitochondrial tumor suppressor, and autophagy under the hypoxia microenvironment. Autophagy. 2012;8:1407–9.

84. Pandhare J, Cooper SK, Phang JM. Proline oxidase, a proapoptotic gene, is induced by troglitazone: evidence for both peroxisome proliferator-activated receptor gamma-dependent and -independent mechanisms. J Biol Chem. 2006;281:2044–52.

85. Fang H, Du G, Wu Q, Liu R, Chen F, Feng J. HDAC inhibitors induce proline dehydrogenase (POX) transcription and anti-apoptotic autophagy in triple negative breast cancer. Acta Biochim Biophys Sin (Shanghai). 2019;51:1064–70.

86. Liu Y, Borchert GL, Surazyński A, Phang JM. Proline oxidase, a p53-induced gene, targets COX-2/PGE2 signaling to induce
apoptosis and inhibit tumor growth in colorectal cancers. Onco-
gen. 2008;27:6729–37.

87. Ding Z, Ericksen RE, Escande-Beillard N, Lee QY, Loh A, Denil S, Steckel M, Haegarbart A, Wai Ho TS, Chow P, Toh HC, Reversade B, Gruenewald S, Han W. Metabolic pathway analyses identify proline biosynthesis pathway as a promoter of liver tumorigenesis. J Hepatol. 2019;72:725.

88. Tanner JJ, Fendt S-M, Becker DP. The proline cycle as a potential cancer therapy target. Biochemistry. 2018;57:3433–44.

89. Craze ML, Cheung H, Jewa N, Coimbra NDM, Soria D, El-
Ansari R, et al. MYC regulation of glutamine–proline regulatory axis is key in luminal B breast cancer. Br J Cancer. 2018;118:258–65.

90. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B. A model for p53-induced apoptosis. Nature. 1997;389:300–5.

91. Myara I, Myara A, Mangeot M, Fabre M, Charpentier C, Lemon-
ier A. Plasma prolidase activity: a possible index of collagen catabolism in chronic liver disease. Clin Chem. 1984;30:211–5.

92. Myara I, Charpentier C, Lemonnier A. Prolidase and prolidase deficiency. Life Sci. 1984;34:1985–98.

93. Mock WL, Green PC, Boyer KD. Specificity and pH depend-
ence for acylproline cleavage by prolidase. J Biol Chem. 1990;265:19600–5.

94. Adibi SA, Mercer DW. Protein digestion in human intestine as reflected in luminal, mucosal, and plasma amino acid concentrations after meals. J Clin Invest. 1973;52:1586–94.

95. Jackson SH, Dennis AW, Greenberg M. Iminodipeptiduria: a genetic defect in recycling collagen: a method for determining prolidase in erythrocytes. Can Med Assoc J. 1975;113(759):62–3.

96. Miltyk W, Palka JA. Potential role of pyrroline 5-carboxylate in regulation of collagen biosynthesis in cultured human skin fibroblasts. Comp Biochem Physiol A Mol Integr Physiol. 2000;125:265–71.

97. Miltyk W, Karna E, Palka J. Inhibition of prolidase activity by non-steroid antiinflammatory drugs in cultured human skin fibro-
blasts. Pol J Pharmacol. 1996;48:609–13.

98. Palka JA, Miltyk W, Karna E, Wolczyński S. Modulation of pro-
lidase activity during in vitro aging of human skin fibroblasts

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.