A New Twist to Ibuprofen: Alternative Action in Alternative Splicing

Authors: *Peter Jordan,[1,2] Vânia Gonçalves,[1,2] Paulo Matos[1,2,3]

1. Department of Human Genetics, National Health Institute 'Dr Ricardo Jorge', Lisbon, Portugal
2. Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal
3. Department of Chemistry and Biochemistry, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

*Correspondence to peter.jordan@insa.min-saude.pt

Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The work undertaken in the authors' laboratory was supported by Fundação para a Ciência e Tecnologia (FCT) through a grant (UID/MULTI/04046/2019) made to the research unit BioISI and contract 'FCT Investigator' to Dr Matos.

Received: 31.07.18

Accepted: 12.02.19

Keywords: Alternative splicing, chemoprevention, colorectal cancer, ibuprofen, nonsteroidal anti-inflammatory drugs (NSAID), RAC1.

Citation: EMJ. 2019;4[2]:64-71.

Abstract

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) and is a widely used medication. One indication of NSAID use is long-term chemoprevention to decrease the risk of developing various types of cancer, in particular colorectal cancer. The molecular mechanism behind the antitumour properties of NSAID has been largely attributed to inhibition of the enzyme cyclooxygenase. In this review article, the authors highlight that additional mechanisms of NSAID, especially ibuprofen, action exist that are related to cell signalling and the modulation of gene expression, including alternative splicing. For example, the authors describe how ibuprofen inhibits expression of the tumour-related splicing variant RAC1b, which is overexpressed in a specific subset of colorectal tumours. The mechanism involves changes in the phosphorylation of splicing factors that regulate this alternative splicing event. According to recent studies, ibuprofen interferes with signal transmission via protein kinases, a process which is frequently altered in cancer cells.

INTRODUCTION

Ibuprofen belongs to the group of nonsteroidal anti-inflammatory drugs (NSAID) used to treat diverse inflammatory processes, pain, or fever. The mechanism underlying the effects of ibuprofen stems from the inhibition of cyclooxygenase (COX) activity, which is required for prostaglandin (PG) synthesis.[1] PG are produced from plasma membrane-derived arachidonic acid and local PG production has hormone-like effects. Two COX isoforms are expressed in human tissues: the constitutively expressed COX-1 isoform exists in most tissues while the COX-2 isoform is strongly induced during the inflammatory response, including pathological conditions of chronic inflammation and colon cancer.[2] Among

different COX-2-derived products, the highest PGE2 levels are found in tumours and affect various processes, including cell proliferation and apoptosis.[3] In normal physiology, PGE2 plays a role in the maintenance of the gastrointestinal mucosa regulating processes, such as mucus secretion and blood vessel dilation.[4] Thus, prolonged NSAID treatment can lead to side effects, including intestinal bleeding. Most NSAID, including ibuprofen, inhibit both COX isoforms.

Prophylactic use of NSAID has been documented to reduce the risk of dying from colorectal cancer.[5-9] For example, a 300 mg daily dose of aspirin over a period of 10 years revealed a statistically significant protective effect.[7,10,11] A similar risk reduction was reported with a daily ibuprofen dose of 200 mg[8,11-19] for various tumour types: 51% reduction in risk for colon, 72% for breast, 62% for prostate, and 59% for lung cancer.[19]

HOW DOES IBUPROFEN PREVENT CANCER?

Accumulating evidence has revealed that inflammation promotes tumourigenesis,[20,21] in particular when the tissue is under chronic inflammatory conditions. Within the tumour microenvironment, inflammatory cells exchange signals with tumour cells. Stromal cells secrete survival factors for tumour cells while tumour cells produce cytokines, which trigger the proteolytic remodelling of the extracellular matrix by stromal cells, or the formation of new blood vessels.[20,22] Ibuprofen inhibits COX activity and the subsequent generation of proinflammatory PG; this action is thought to underlie the chemopreventive effect of ibuprofen. PGE2, for example, activates G protein-coupled PGE2 receptors that stimulate various signalling pathways involved in cell proliferation and survival.[23,24]

In this article, the authors review additional mechanisms of action that are independent of COX-2 inhibition with the aim of increasing awareness that the clinical effects of ibuprofen can be mediated by several cellular processes. The presented evidence was retrieved from the PubMed search engine using "ibuprofen AND cancer" as the search term. Studies reporting COX-independent effects, including those conducted in the authors' laboratory, were selected for review.

ADDITIONAL MECHANISMS THROUGH WHICH IBUPROFEN INHIBITS TUMOUR CELLS

In 2015, Matos and Jordan[25] reviewed the treatment of cancer cells with ibuprofen. HCT-116 colorectal cells do not express COX-2, but the treatment with 2 mMol/L ibuprofen produced proapoptotic effects.[26] Ibuprofen at a low concentration of 100 µMol was further identified as a direct and COX-independent ligand of peroxisome proliferator-activated receptor gamma (PPARγ),[27] and was shown to stimulate its nuclear activity in rat models of colon cancer formation.[28] Thus, the proapoptotic action observed for ibuprofen may in part result from PPARγ activation, which leads to the downregulation of the antiapoptotic transcription factor NFκB.[28]

Another COX-independent cellular response following ibuprofen treatment was reported to involve $P75^{NTR}$, a member of the TNF receptor superfamily. Treatment of cancer cells with 1 mMol/L ibuprofen resulted in a p38 mitogen-activated protein kinase pathway-dependent stabilisation of $p75^{NTR}$ mRNA stability, leading to increased expression levels[29] and induction of apoptosis and growth suppression.[30]

A similar apoptosis-promoting action was reported in HCT116 cells, when ibuprofen treatment (1.5 mM for 24 hours) was found to sensitise these cells against the TNF-related apoptosis-inducing ligand.[31] The underlying mechanism involved expression of the membrane receptor for TNF-related apoptosis-inducing ligand: death receptor 5, another member of the TNF receptor superfamily.

Ibuprofen treatment (1 mMol/L for 24 hours) was further reported to significantly reduce the nuclear levels of β-catenin in SW480 and DLD-1 colorectal tumour cells. Consistently, the expression of one of its transcriptional targets, the pro-proliferative cyclin D1 gene, was suppressed.[32] Although the underlying mechanism remains to be determined, this effect of ibuprofen seems of special interest for

colorectal cancer prevention because excessive β-catenin signalling can cause inappropriate growth stimulation of colon mucosa stem cells.[33]

Concurrent to the effect on β-catenin signalling, ibuprofen also interfered directly with the NFκB pathway. A rapid effect of ibuprofen treatment observed in cells is the inhibitory phosphorylation of GSK-3β at serine 9.[32] This modification was found to negatively regulate NFκB signalling, at a step downstream of the degradation of its inhibitor protein IkBα, and to suppress the expression of anti-apoptotic NFκB target genes, such as *BCL2* and *BIRC5*.

Other examples for COX-independent effects of 100 µMol ibuprofen include the inhibition of integrin expression in neutrophils[34] or the caspase-mediated release of proinflammatory cytokines in HCT-116 and HeLa cells.[35]

IBUPROFEN, ALTERNATIVE SPLICING, AND CANCER

Cancer cells differ in their gene expression programme from their corresponding differentiated normal cells. Besides transcriptional regulation at gene promoters, the past 15 years have clearly revealed that alternative splicing serves as a significant mechanism for the regulation of gene expression. For example, alternative splicing generates transcript variants that can either be non-functional and rapidly degraded or be translated into protein isoforms with different, sometimes antagonistic, functional properties due to differential use of functional protein domains.[36,37]

Recently, inhibition of the alternative splicing variant RAC1b was identified as another COX-independent effect of ibuprofen.[38] Colon inflammation was shown as one trigger for increased expression of the tumour-related RAC1b protein, a splice variant of the small GTPase RAC1. RAC1b protein contains an additional domain encoded by a 57 base pair-long alternative exon (exon 3b), which confers increased protein activation, generating a hyperactive variant able to stimulate NFκB signalling.[39-42] When colorectal cells were treated with ibuprofen, but not with aspirin or flurbiprofen, both the mRNA and protein levels of RAC1b were markedly reduced *in vitro* and *in vivo*.[38] Whereas many studies on the effect of NSAID on tumour cell viability used concentrations of up to 2 mMol/L,[43] the effect of ibuprofen on alternative splicing of RAC1b was observed at low doses of 100 µMol. Interestingly, ibuprofen inhibited RAC1b-positive HT29 colorectal cells more than normal colonocytes and also affected their growth as subcutaneous tumour xenografts in mice. The inhibitory effect of ibuprofen could be rescued when a splicing-independent RAC1b cDNA sequence was expressed in HT29 cell.[38] This suggests that ibuprofen acts directly on the alternative splicing event.

Another report on the modulation of alternative splicing was obtained when prostate cancer cells received combined treatment of ibuprofen and epigallocatechin-3-gallate (EGCG), a green tea component with anticarcinogenic properties that promotes G0/G1 cell cycle arrest and apoptosis. In this case, the balance between anti and proapoptotic splicing variants of BCL-X and MCL-1 was shifted towards the shorter and proapoptotic BCL-X(S) or MCL-1(S) variants.[44] Although the mechanism was not fully identified, it involves activation of protein phosphatase PP1, which is known to dephosphorylate regulatory proteins involved in pre-mRNA splicing.

MECHANISM OF SPLICING MODULATION BY IBUPROFEN

When protein-coding genes are expressed in human cells, RNA polymerase 2 generates a primary transcript, the pre-mRNA, which contains coding exons separated by intronic sequences. While transcription is ongoing, conserved nucleotide sequences around each exon–intron junction are recognised by the spliceosome, a macromolecular machinery involving five small nuclear ribonucleoprotein particles (U1, U2, U4, U5, and U6 small nuclear ribonucleoprotein),[45,46] which then removes introns during the process of mRNA splicing. The function of the spliceosome is assisted by splice enhancer or silencer elements, short sequences found in exons or introns, which either promote or inhibit productive recognition of a given exon by the spliceosome. Splicing factors recognise these splice enhancer or silencer elements, which mostly belong to the serine and arginine rich protein family or the heterogeneous nuclear ribonucleoproteins. They often act antagonistically, so that the modulation of

binding provides a mechanism that allows inclusion or skipping of alternative exon and thus the generation of variant transcripts. Altogether, the set of splicing factors expressed in a given cell and their relative expression levels in the cell nucleus operate in a combinatorial mode to regulate alternative splicing.

In the case of RAC1b, alternative splicing is regulated by an enhancer element in exon 3b, which is recognised by the splicing factor SRSF1, and an adjacent silencer element recognised by SRSF3.[47]

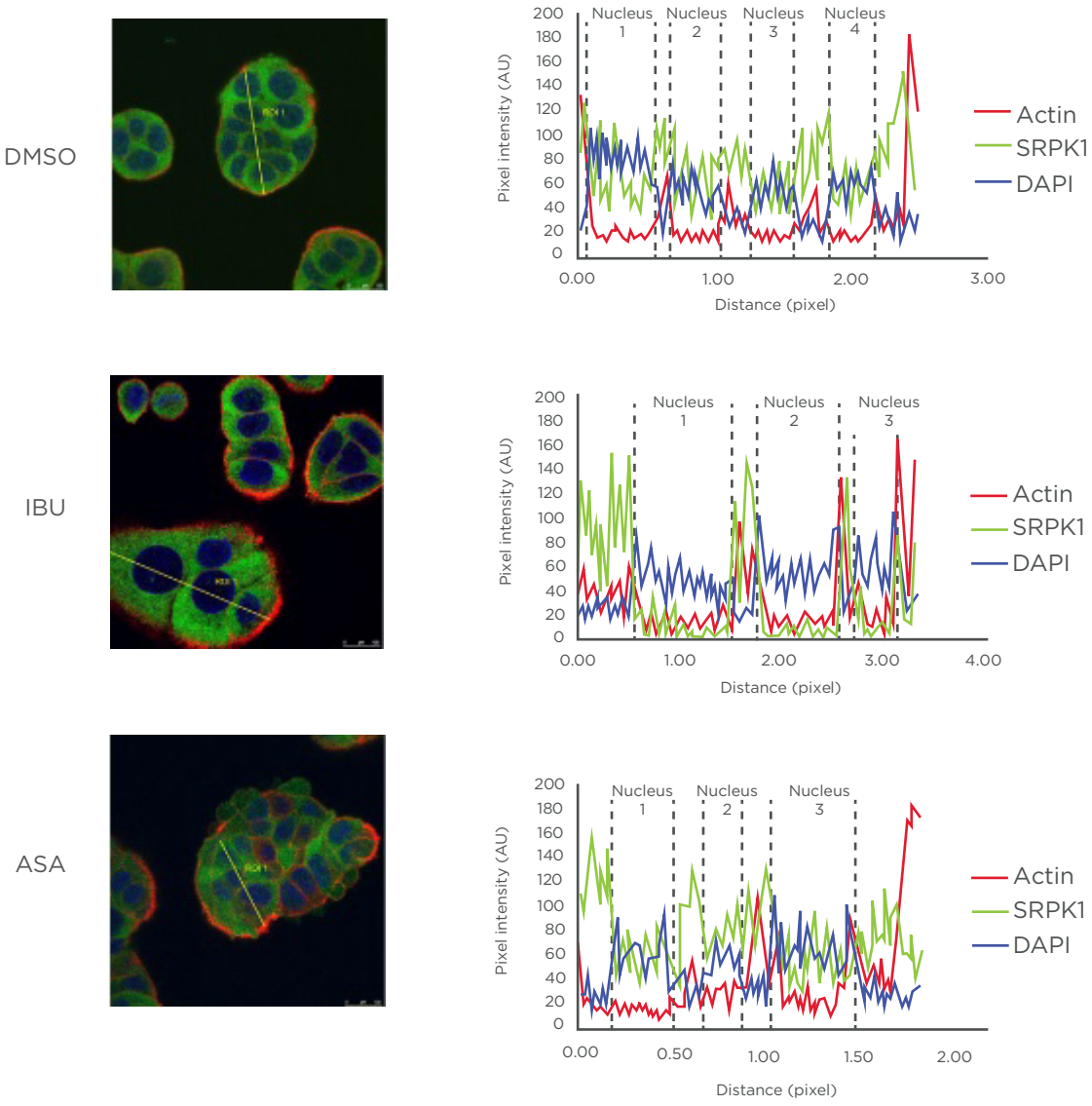

Figure 1: Effect of ibuprofen on subcellular localisation of protein kinase SRPK1.

HT29 colorectal cells were incubated for 48 hours with either a DMSO control solvent, 500 µM ibuprofen, or 500 µM aspirin and then fixed for immunofluorescence microscopy. Shown is the coloured overlay of three confocal immunofluorescence images (left), which detected cell nuclei in blue (DAPI), the localisation of endogenous SRPK1 protein in green (anti-SRPK1, BD Biosciences, San Jose, California, USA), and the actin cytoskeleton in red (Phalloidin-Texas Red). The nucleus and cytoplasm distribution of the three fluorescent signals was analysed along optical sections (yellow lines) across several cells by plotting pixel intensities along the traced path (right graphs). In control and aspirin-treated cells, SRPK1 signals (green) were localised both to the cytosol and the cell nucleus (blue); however, in ibuprofen-treated cells, nuclear signals for SRPK1 are nearly absent.

AU: arbitrary units; ASA: aspirin; DAPI: 4′,6-diamidino-2-phenylindole; DMSO: dimethyl sulfoxide; IBU: ibuprofen; SRPK1: serine/threonine-protein kinase.

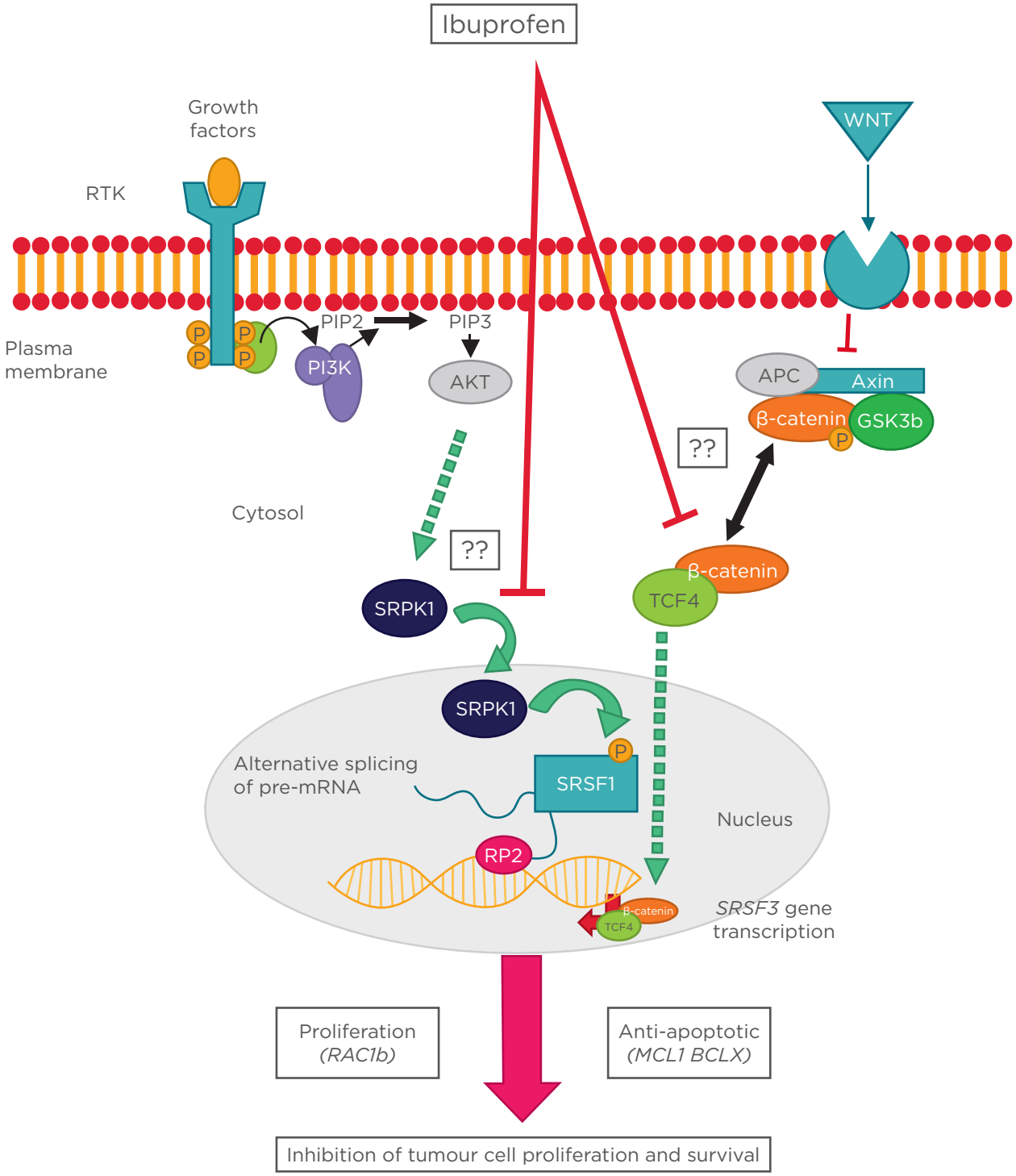

Figure 2: Schematic representation of the cellular pathways linking ibuprofen to the cyclooxygenase-independent modulation of alternative splicing.

Following stimulation of receptor tyrosine kinases, the PI3K becomes activated and leads to phosphorylation of SRPK1, which enters the nucleus and phosphorylates the splicing factor SRSF1. SRSF1 binds to specific recognition motifs on nascent pre-mRNA transcribed by RNA polymerase 2, thus affecting alternative splicing decisions. In a parallel pathway, Wnt ligands stimulate their plasma membrane receptor leading to inhibition of the β-catenin destruction complex and accumulation of a cytosolic β-catenin/TCF4 complex. This complex enters the nucleus and binds to gene promoters, including that of splicing factor SRSF3, the expression levels of which determine the outcome of specific splicing events. Examples of splicing variants affected by these pathways are *RAC1b*, *MCL1*, and *BCLX*. Question marks indicate that the molecular mechanism is still unknown.

AKT: protein kinase B; APC: adenomatous polyposis coli; GSK: glycogen synthase kinase; MCL1: myeloid Cell Leukemia Sequence 1; PIP2: phosphatidylinositol 4,5-bisphosphate; PIP3: phosphatidylinositol (3,4,5)-trisphosphate; PI3K: phosphoinositide 3-kinases; RAC1: Ras-related C3 botulinum toxin substrate 1: RP2: RNA polymerase 2; RTK: receptor tyrosine kinases; SRPK1: serine/threonine-protein kinase; SRSF: serine and arginine rich splicing factor; TCF4: transcription factor 4; Wnt: wingless/integrated.

In human colorectal cells, the availability of SRSF1 in the nucleus is the main factor regulating inclusion or skipping of exon 3b.[48]

One mechanism through which ibuprofen does affect alternative splicing in cells is the phosphorylation status of SRSF1. Cell fractionation and immunoblot experiments revealed that ibuprofen treatment caused a reduction in SRSF1 phosphorylation (unpublished data). By contrast, aspirin treatment had no such effect on SRSF1. This showed that the inhibitory effect of ibuprofen on RAC1b splicing involved post-translational regulation of SRSF1 subcellular localisation.[48]

The main protein kinase responsible for SRSF1 phosphorylation is SRPK1, which is found both in the cytoplasm and in the cell nucleus.[49,50] This process is, in part, controlled by growth factor receptor signalling.[51] As shown and described in Figure 1, the authors observed that ibuprofen treatment induced translocation of SRPK1 from the nucleus into the cytoplasm, and this correlated with reduced levels of SRSF1 phosphorylation and RAC1b protein as detected in whole cell lysates by western blot. No such effect was observed when cells were treated with aspirin under the same conditions, underlining the COX-independent action of ibuprofen and the specificity of its effect on splicing factor modulation.

Another mechanism through which ibuprofen can regulate splicing is the transcriptional modulation of splicing factor-encoding genes. The splicing factor SRSF3, for example, was previously described to be a direct transcriptional target for β-catenin/TCF signalling and ibuprofen has been found to downregulate β-catenin/TCF signalling in colorectal cells.[52] A reduction in SRSF3 transcription and the consequent decrease in its nuclear levels will affect a variety of splicing variants.

Further research may unravel that, besides SRSF1 and SRSF3, other splicing factors are also modulated by ibuprofen treatment, either by regulation of their expression levels, their subcellular localisation, or their RNA-binding activity. These effects will most likely also include COX-dependent mechanisms as many of the PGE2 stimulated pathways[23,24] have been described to affect alternative splicing regulation.[53] It can thus be expected that ibuprofen treatment will affect a larger set of alternative splicing events in cancer cells and that these contribute to the described antiproliferative and proapoptotic effects.

CONCLUSION

Although ibuprofen has been used for chemopreventive therapies against cancers in the gastrointestinal tract, our understanding of the molecular mechanisms underlying the antineoplastic activity of ibuprofen is still rudimentary. Recently described cellular pathways linking ibuprofen to the COX-independent modulation of alternative splicing are summarised in Figure 2. A better characterisation of its target molecules and their signalling pathways may provide opportunities for precision medicine approaches in cancer therapy or chemoprevention regimens. For example, the inhibitory effect on alternative splicing of *RAC1b* may benefit a subgroup of colorectal cancer patients characterised by serrated polyp morphology, *BRAF* mutation, and RAC1b overexpression. However, the deregulation of splicing factor SRSF1, which was described in this case, is most likely only the tip of the iceberg. It is now known that deregulation of splicing factors will inevitably affect a network of alternative splicing changes and this can be expected to have significant impact on cancer cell biology. A more systematic study with genome-wide determination of transcriptome changes should clarify the therapeutic opportunities that may arise from the COX-independent action of ibuprofen.

References

1. Vane JR, Botting RM. Mechanism of action of antiinflammatory drugs. Int J Tissue React. 1998;20(1):3-15.
2. Terzić J et al. Inflammation and colon cancer. Gastroenterology. 2010;138(6):2101-14.e5.
3. Sobolewski C et al. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. Int J Cell Biol. 2010;2010:1-21.
4. Nakanishi M, Rosenberg DW. Multifaceted roles of PGE2 in inflammation and cancer. Semin Immunopathol. 2013;35(2):123-37.
5. Chan TA. Nonsteroidal anti-inflammatory drugs, apoptosis, and colon-cancer chemoprevention. Lancet Oncol. 2002;3(3):166-74.
6. Thun MJ et al. Nonsteroidal anti-inflammatory drugs as anticancer agents: Mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst. 2002;94(4):252-66.
7. Chan AT et al. Long-term use of aspirin and nonsteroidal anti-inflammatory drugs and risk of colorectal cancer. JAMA. 2005;294(8):914-23.
8. Johnson CC et al.; PLCO Trial Team. Non-steroidal anti-inflammatory drug use and colorectal polyps in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Am J Gastroenterol. 2010;105(12):2646-55.
9. Ruder EH et al. Non-steroidal anti-inflammatory drugs and colorectal cancer risk in a large, prospective cohort. Am J Gastroenterol. 2011;106(7):1340-50.
10. García-Rodríguez LA, Huerta-Alvarez C. Reduced risk of colorectal cancer among long-term users of aspirin and nonaspirin nonsteroidal antiinflammatory drugs. Epidemiology. 2001;12(1):88-93.
11. Brasky TM et al. Non-steroidal anti-inflammatory drugs and cancer incidence by sex in the VITamins And Lifestyle (VITAL) cohort. Cancer Causes Control. 2012;23(3):431-44.
12. Baron JA, Sandler RS. Nonsteroidal anti-inflammatory drugs and cancer prevention. Annu Rev Med. 2000;51:511-23.
13. García Rodríguez LA, Huerta-Alvarez C. Reduced incidence of colorectal adenoma among long-term users of nonsteroidal antiinflammatory drugs: A pooled analysis of published studies and a new population-based study. Epidemiology. 2000;11(4):376-81.
14. Harris RE et al. Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: A critical review of non-selective COX-2 blockade (review). Oncol Rep. 2005;13(4):559-83.
15. Harris RE et al. Similar reductions in the risk of human colon cancer by selective and nonselective cyclooxygenase-2 (COX-2) inhibitors. BMC Cancer. 2008;8:237.
16. Zell JA et al. Meat consumption, nonsteroidal anti-inflammatory drug use, and mortality among colorectal cancer patients in the California Teachers Study. Cancer Prev Res (Phila Pa). 2010;3(7):865-75.
17. Zhang Y et al. Use of nonsteroidal antiinflammatory drugs and risk of breast cancer: The Case-Control Surveillance Study revisited. Am J Epidemiol. 2005;162(2):165-70.
18. Kwan ML et al. NSAIDs and breast cancer recurrence in a prospective cohort study. Cancer Causes Control. 2007;18(6):613-20.
19. Harris R et al. Reduction in cancer risk by selective and nonselective cyclooxygenase-2 (COX-2) inhibitors. J Exp Pharmacol. 2012;4:91-6.
20. Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002;420(6917):860-7.
21. Bissell MJ, Hines WC. Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat Med. 2011;17(3):320-9.
22. DiDonato JA et al. NF-κB and the link between inflammation and cancer. Immunol Rev. 2012;246(1):379-400.
23. Greenhough A et al. The COX-2/PGE2 pathway: Key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. Carcinogenesis. 2009;30(3):377-86.
24. Su CW et al. Stromal COX-2 signaling are correlated with colorectal cancer: A review. Crit Rev Oncol Hematol. 2016;107:33-8.
25. Matos P, Jordan P. Beyond COX-inhibition: "Side-effects" of ibuprofen on neoplastic development and progression. Curr Pharm Des. 2015;21(21):2978-82.
26. Khwaja F et al. Ibuprofen inhibits survival of bladder cancer cells by induced expression of the p75NTR tumor suppressor protein. Cancer Res. 2004;64(17):6207-13.
27. Lehmann JM et al. Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem. 1997;272(6):3406-10.
28. Vaish V et al. The role of NF-κB and PPARγ in experimentally induced colorectal cancer and chemoprevention by cyclooxygenase-2 inhibitors. Tumor Biol. 2010;31(5):427-36.
29. Quann EJ et al. The p38 MAPK pathway mediates aryl propionic acid induced messenger RNA stability of p75 NTR in prostate cancer cells. Cancer Res. 2007;67(23):11402–10.
30. Quann EJ et al. The aryl propionic acid R-flurbiprofen selectively induces p75NTR-dependent decreased survival of prostate tumor cells. Cancer Res. 2007;67(7):3254-62.
31. Todo M et al. Ibuprofen enhances TRAIL-induced apoptosis through DR5 upregulation. Oncol Rep. 2013;30(5):2379-84.
32. Greenspan EJ et al. Ibuprofen inhibits activation of nuclear β-catenin in human colon adenomas and induces the phosphorylation of GSK-3β. Cancer Prev Res (Phila Pa). 2011;4(1):161-71.
33. Qiu W et al. Chemoprevention by nonsteroidal anti-inflammatory drugs eliminates oncogenic intestinal stem cells via SMAC-dependent apoptosis. Proc Natl Acad Sci U S A. 2010;107(46):20027-32.
34. Bertolotto M et al. Neutrophil migration towards C5a and CXCL8 is prevented by non-steroidal anti-inflammatory drugs via inhibition of different pathways: NSAIDs and neutrophil migration. Br J Pharmacol. 2014;171(14):3376-93.
35. Smith CE et al. Non-steroidal anti-inflammatory drugs are caspase inhibitors. Cell Chem Biol. 2017;24(3):281-92.
36. David CJ, Manley JL. Alternative pre-mRNA splicing regulation in cancer: Pathways and programs unhinged. Genes Dev. 2010;24(21):2343-64.
37. Buljan M et al. Tissue-specific splicing of disordered segments that embed binding motifs rewires protein interaction networks. Mol Cell. 2012;46(6):871-83.
38. Matos P et al. Ibuprofen inhibits colitis-induced overexpression of tumor-related Rac1b. Neoplasia. 2013;15(1):102-11.
39. Matos P et al. Tumor-related alternatively spliced Rac1b is not regulated by Rho-GDP dissociation inhibitors and exhibits selective downstream signaling. J Biol Chem. 2003;278(50):50442-8.
40. Matos P, Jordan P. Increased Rac1b expression sustains colorectal tumor cell survival. Mol Cancer Res MCR. 2008;6(7):1178-84.
41. Matos P, Jordan P. Expression of Rac1b stimulates NF-kappaB-mediated cell survival and G1/S progression. Exp Cell Res. 2005;305(2):292-9.
42. Matos P et al. B-Raf(V600E) cooperates with alternative spliced Rac1b to sustain colorectal cancer cell survival. Gastroenterology. 2008;135(3):899-906.

43. Gurpinar E et al. NSAIDs inhibit tumorigenesis, but how? Clin Cancer Res. 2014;20(5):1104-13.
44. Kim MH. Protein phosphatase 1 activation and alternative splicing of Bcl–X and Mcl–1 by EGCG + ibuprofen. J Cell Biochem. 2008;104(4):1491-9.
45. Matera AG, Wang Z. A day in the life of the spliceosome. Nat Rev Mol Cell Biol. 2014;15(2):108-21.
46. Wahl MC et al. The spliceosome: Design principles of a dynamic RNP machine. Cell. 2009;136(4):701-18.
47. Gonçalves V et al. Antagonistic SR proteins regulate alternative splicing of tumor-related Rac1b downstream of the PI3-kinase and Wnt pathways. Hum Mol Genet. 2009;18(19):3696-707.
48. Gonçalves V et al. Phosphorylation of SRSF1 by SRPK1 regulates alternative splicing of tumor-related Rac1b in colorectal cells. RNA. 2014;20(4):474-82.
49. Zhong XY et al. Regulation of SR protein phosphorylation and alternative splicing by modulating kinetic interactions of SRPK1 with molecular chaperones. Genes Dev. 2009;23(4):482-95.
50. Ding JH. Regulated cellular partitioning of SR protein-specific kinases in mammalian cells. Mol Biol Cell. 2005;17(2):876-85.
51. Zhou Z et al. The Akt-SRPK-SR axis constitutes a major pathway in transducing EGF signaling to regulate alternative splicing in the nucleus. Mol Cell. 2012;47(3):422-33.
52. Gonçalves V et al. The beta-catenin/TCF4 pathway modifies alternative splicing through modulation of SRp20 expression. RNA. 2008;14(12):2538-49.
53. Gonçalves V et al. Signaling pathways driving aberrant splicing in cancer cells. Genes. 2017;9(1).