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Review

Chemopreventive effects of aspirin at a glance

Muhammad Waqas Usman [a,b,1], Fuwen Luo [c,1], Hailing Cheng [a,d,e,*], Jean J. Zhao [d,e,f,**], Pixu Liu [a,b,d,***]

[a] Cancer Institute, Second Affiliated Hospital, Dalian Medical University, Dalian, Liaoning, China
[b] Institute of Cancer Stem Cell, Dalian Medical University, Dalian, Liaoning, China
[c] Department of Acute Abdomen Surgery, Second Affiliated Hospital, Dalian Medical University, Dalian, Liaoning, China
[d] Department of Cancer Biology, Dana–Farber Cancer Institute Harvard Medical School, Boston, MA, USA
[e] Department of Surgery, Brigham and Women's Hospital Harvard Medical School, Boston, MA, USA
[f] Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

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ABSTRACT

Experimental, epidemiological, and clinical data from the last two decades have each supported the hypothesis that aspirin possesses anticancer properties, and that its use may also reduce the lifetime probability of developing or dying from a number of cancers. Aspirin's ability to act on multiple key metabolic and signaling pathways via inhibition of the cyclooxygenase (COX) enzyme, as well as through COX-independent mechanisms, makes it particularly relevant in the fight against cancer. A growing body of evidence indicates that aspirin may not only reduce cancer risk, but also prevent metastasis and angiogenesis while slowing the rate of mutation-inducing DNA damage. These emerging benefits of aspirin are offset to some extent by the known risks of treatment, such as cardiovascular events and gastrointestinal bleeding. However, it has been shown that pre-treatment risk assessment of individual patients and the use of proton pump inhibitors or *Helicobacter pylori* eradication therapy concomitantly with aspirin treatment can reduce these potential risks. Thus, the significant benefits of aspirin treatment, coupled with recent data concerning its risks, may prove to tip the balance in favor of aspirin use in cancer prevention.

Contents

1. Background

Aspirin is a widely used, inexpensive drug that is easily available without any prescription. Its story began in the late 18th century with the use of willow bark extract containing salicylate as an analgesic and antipyretic agent. With few modifications, this ancient remedy was first given the name aspirin in 1897 by German Chemist Felix Hoffman [1]. Decades later, in 1971, Sir John Vane elucidated aspirin's active mechanism as an inhibitor of prostaglandin synthetase [2] through the irreversible acetylation of a serine residue at position 529 [3]. Today, the prevalence of aspirin use has significantly increased as it has also

* Correspondence to: H. Cheng, Cancer Institute, Second Affiliated Hospital, Dalian Medical University, Dalian, Liaoning, China.
** Correspondence to: J.J. Zhao, Dana Farber Cancer Institute, Harvard Medical School, 450 Brookline Avenue, Smith 936, Boston, MA 02215, USA. Tel.: +1 617 632 2932.
*** Correspondence to: P. Liu, Institute of Cancer Stem Cell, Dalian Medical University, Dalian, Liaoning 116044, China. Tel.: +86 15840970870.
E-mail addresses: hailing_cheng@dfci.harvard.edu (H. Cheng), Jean_zhao@dfci.harvard.edu (J.J. Zhao), Pixu_liu@dlmedu.edu.cn (P. Liu).
[1] These authors contributed equally to this work.

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been shown to reduce the risk of myocardial infarction and stroke [4,5]. In recent years, emerging evidence showing the benefits of aspirin in cancer prevention has ignited a renewed interest in its research [6]. In this article, we review the recent literature concerning the role of aspirin in cancer prevention and highlight its influence on several key hallmarks of cancer as a disease. Furthermore, we consider the potential mechanism of aspirin in cancer prevention and its ability to target several signaling pathways. This review also presents some important insights into the benefits and risks of aspirin use as related to its dose and the duration of treatment, which may prove to be helpful in forming a future clinical standard of aspirin use for cancer prophylaxis.

2. Aspirin as a chemopreventive agent

In the last decade, multiple new lines of evidence from a rapidly growing body of research have strengthened the hypothesis that aspirin

Table 1
Effects of aspirin on overall and site-specific cancer incidence and mortality.

Study	Follow-up/exposure period	Definition of aspirin use	Case/control or subject	HR or RR, 95% CI	Reference
Cancer incidence					
Women Health Initiative Observational Study and Clinical Trial	Median follow-up period of 9.7 years	Any aspirin used for ≥5 years	496/3743	HR = 0.98, 95% CI (0.89 to 1.08)[a] overall cancer	Brasky et al. [23]
			82/5859	HR = 0.73, 95% CI (0.54 to 0.97)[b] gastrointestinal cancer	
			54/5887	HR = 0.69, 95% CI (0.48 to 0.99)[b] colorectal cancer	
			72/5869	HR = 0.89, 95% CI (0.65 to 1.22)[a] lung cancer	
			244/5697	HR = 1.05, 95% CI (0.89 to 1.23)[a] breast cancer	
			8/4824	HR = 0.37, 95% CI (0.16 to 0.84)[b] ovarian cancer	
			24/5917	HR = 0.69, 95% CI (0.40 to 1.20)[c] melanoma	
Cancer Prevention Study II Nutrition Cohort	Median follow-up period of 10 years	Daily use of adult-strength aspirin (≥325 mg/day) for ≥5 years	493/23,259	RR = 0.84, 95% CI (0.76 to 0.93)[b] male (overall cancer)	Jacobs et al. [7]
			146/12,427	RR = 0.86, 95% CI (0.73 to 1.03)[c] female (overall cancer)	
			225/24,033	RR = 0.81, 95% CI (0.70 to 0.94)[b] prostate cancer	
			55/12,721	RR = 0.83, 95% CI (0.63 to 1.10)[c] breast cancer	
			60/38,302	RR = 0.68, 95% CI (0.52 to 0.90)[b] colorectal cancer	
			85/38,400	RR = 0.98, 95% CI (0.76 to 1.25)[a] lung cancer	
Women Health Study	Average follow-up of 10.1 years	100 mg of aspirin administered every other day	2865/39,876	RR = 1.01, 95% CI (0.94 to 1.08)[a] overall cancer	Cook et al. [24]
			1230/39,876	RR = 0.98, 95% CI (0.87 to 1.09)[a] breast cancer	
			269/39,876	RR = 0.97, 95% CI (0.77 to 1.24)[a] colorectal cancer	
			205/39,876	RR = 0.78, 95% CI (0.59 to 1.03)[c] lung cancer	
			205/39,876	RR = 0.95, 95% CI (0.68 to 1.35)[a] ovarian cancer	
Cancer mortality					
Long Term Effect of Aspirin on Cancer Mortality	20 years follow up	Daily use of aspirin for ≥5 years	1378/10,502	HR = 0.78, 95% CI (0.70 to 0.87)[b] overall cancer	Rothwell et al. [8]
			126/NS	HR = 1.09, 95% CI (0.76 to 1.56)[a] hematological cancers	
			179/NS	HR = 0.60, 95% CI (0.45 to 0.81)[b] colorectal cancer	
			210/NS	HR = 0.81, 95% CI (0.61 to 1.06)[a] prostate cancer	
			326/NS	HR = 0.71, 95% CI (0.58 to 0.89)[b] lung cancer	
			62/NS	HR = 0.42, 95% CI (0.25 to 0.71)[b] esophageal cancer	
Women Health Study	Average follow-up of 10.1 years	100 mg of aspirin administered every other day for ≥5 years	583/39,876	RR = 0.98, 95% CI (0.89 to 1.09)[a] overall cancer	Cook et al. [24]
			65/39,876	RR = 0.96, 95% CI (0.78 to 1.18)[a] colorectal cancer	
			140/39,876	RR = 0.68, 95% CI (0.45 to 1.05)[c] lung cancer	
			63/39,876	RR = 0.96, 95% CI (0.83 to 1.12)[a] breast cancer	

Abbreviations: NS, not specified; CI, confidence interval; HR, hazards ratios; RR, relative risk.
[a] No reduction.
[b] Statistically significant reduction.
[c] Statistically non-significant reduction.

may help to prevent certain cancers from occurring and reduce the overall risk of dying from cancer [6,7]. However, the effect of aspirin on overall and site-specific cancer incidence and mortality was not uniform among several large cohort and case–control studies (as shown in Table 1). Of particular note, Lancet recently published the results of a large observational study that was comprised of 8 trials with more than 25,000 individuals, and which showed that taking a daily low dose of aspirin reduced the long-term mortality rate from a number of common cancers [8].

The strongest evidence for the use of aspirin in cancer prevention thus far lies in the data concerning aspirin's potential protective effects against colon cancer. Regular use of adult-strength (375 mg) aspirin was associated with a 20% statistically significant reduced risk of colorectal cancer [9], and taking low-dose aspirin every other day also appears to have a lesser protective effect against colon cancer [10]. However, the use of low-dose aspirin following the diagnosis of colorectal cancer does not improve the overall survival rate [11]. The association between improved overall survival rate and low-dose aspirin use still remains unclear, with many studies published in this regard reporting conflicting results. These studies have linked the efficacy of low-dose aspirin use in cancer prevention with multiple factors, including PIK3CA mutation status, PTGS2 overexpression [12], high expression of HLA Class I antigen [13] and 15-hydroxyprostaglandin dehydrogenase enzyme [14], and single nucleotide polymorphism in rs6983267 genotype [15].

In addition to its chemopreventive effects on colorectal cancers, aspirin has also been shown to have a beneficial role in reducing lung cancer incidence and mortality [8]. A pooled analysis conducted by the International Lung Cancer Consortium (ILCCO) suggests that aspirin use was significantly associated with reduced risk of lung cancer [16] regardless of the smoking status of the individual [17]. Of further interest, a study published within the last year suggests that aspirin use may also reduce metastasis of lung cancer cells to the regional lymph nodes, and thus has the potential to extend the lives of lung cancer patients [18]. However, a meta-analysis published in Annals of Oncology, contradicts the above finding, as even though the preventive effect of aspirin was observed in case–control studies, the higher-level evidence of cohort studies was shown to outweigh the preventive effect of aspirin seen in case–control studies [19].

The risk of esophageal cancer may also be reduced through the use of preventive aspirin therapy, particularly among patients with gastroesophageal reflux [20]. Furthermore, low dose aspirin also confers a significant protective effect against developing esophageal adenocarcinoma in patients with Barrett's esophagus [21]. Multiple meta-analyses published within the last two years demonstrate that aspirin use confers a significant protective effect against esophageal cancer, and additionally suggest that the degree of protection may be increased by longer treatment duration and higher frequency of usage [21,22]. In addition to the preventive effects of aspirin on cancer incidence, low doses of aspirin were also found to reduce the long-term risk of death due to esophageal cancer [8].

The association between aspirin use and breast cancer prevention remains inconclusive. Several large rigorous studies, such as the Women's Health Study and the Women's Health Initiative program [23,24], found no difference in breast cancer incidence among women treated with aspirin or with placebo during 10 years of intervention and follow up [23,24]. However, results from the Iowa Women's Health Study of postmenopausal women revealed that taking a low-dose aspirin every day might have the potential to prevent and reduce breast cancer risk by 20% [25]. Similarly, a recent study found that women with pre-diagnostic aspirin use were less likely to develop node-positive breast cancer [26]. In addition, pre-diagnostic aspirin use was found to be associated with a lower 5-year mortality rate in lymph node-negative breast cancers [26]. A collaborative study conducted last year by Gargi, Maity et al. found that daily low-dose aspirin slowed the growth of breast cancer cell lines, as well as significantly shrank tumors and prevented metastasis in a xenograft mouse model. Most strikingly, these results were obtained using triple-negative breast cancer cell lines, which is among the most difficult forms of breast cancer to treat [27]. Evidence based on sub-group analysis shows that the lowered risk of breast cancer, which was associated with increasing frequency of aspirin use, was independent of the hormone receptor status of the tumors [25].

Other hormone-related cancers have also shown preventative benefits from daily low-dose aspirin use. For example, elderly and middle-aged women who took low-dose aspirin continuously over an extended period of time were less prone to ovarian cancer when compared to age-matched women who did not use low-dose aspirin [28]. A pooled analysis of 12 population-based, case-controlled studies of ovarian cancer showed that this reduction of risk is up to 20% to 34%, depending on the dose, duration, and frequency of aspirin treatment used [29]. In contrast, no significant association was found between aspirin use and ovarian cancer mortality [30]. In addition to a reduced risk of ovarian cancer, several population-based, observational studies have demonstrated that long-term aspirin use may also reduce risk for prostate cancer [31,32]. A recent study conducted by Vidal et al. revealed that the use of aspirin lowered the risk of developing aggressive prostate cancer by 17% [33]. Of note, despite the previously reported link between aspirin efficacy and race or geographic region [31,32], the risk of developing prostate cancer was comparable between European and American patients [33]. As with ovarian cancer, the impact of aspirin use on prostate cancer risk appears to be associated with dose, as men taking high-dose aspirin had a lower risk of prostate cancer-specific mortality, compared with low-dose users [34].

In addition to the cancers discussed above, there are a growing number of studies which suggest that aspirin is also successful in preventing a variety of other solid tumor cancers, including gastric, melanoma, pancreatic, and head and neck cancers, but not hematological cancers [8,23,35,36]. However, these potential anti-cancer effects must also be evaluated in the context of recent research concerning cardiovascular risks of treatment, such as venous thromboembolism, which warrant further exploration for a more comprehensive understanding of the utility of aspirin treatment in chemoprevention [37].

3. Dose and duration of aspirin therapy for cancer prophylaxis

The rapidly growing body of research on this topic has yet to reveal clear evidence to suggest a minimal effective dose and duration of aspirin use that achieves the desired benefits while minimizing the potential harmful effects of the treatment. Evidence from recent studies suggests that the chemopreventive effects of aspirin are attained even at low doses (defined as 75–100 mg), which is similar to the dose required for the cardiovascular benefits of the drug to become evident [8,29,38,39]. However, additional studies suggest that the extent of treatment benefits is further increased with higher-dose aspirin. One such study on colorectal cancer incidence demonstrated that patients taking 6–14 tablets of standard-dose (380 mg) aspirin per week saw more benefits (RR = 0.30, 95% CI, 0.11–0.81) than patients who used only 0.5–1.5 tablets (RR = 0.80, 95% CI, 0.63–1.01) or 2–5 tablets (RR = 0.72, 95% CI, 0.56–0.92), but that this higher dose also doubled the risk of major intestinal bleeding [40,41]. Controversially, two U.S.-based trials, the Physician's Health Study and the Women's Health study, both contradict the above findings. Their results suggest that alternate-day use of 100 mg or 350 mg of aspirin for 5 or 10 years, respectively, is not associated with reduced risk of cancer. However, these studies also indicate that this dose and duration of aspirin treatment did, to some extent, reduce the mortality rate specific to certain cancers [24,42]. In these trials, the effects of aspirin on cancer were not the primary endpoint, and thus the short follow-up period and alternate-day dosing are possible confounding factors which may have masked the chemopreventative benefits of aspirin.

In addition to the impact of dose, several studies also reveal the importance of duration and continuity of aspirin use in attaining chemopreventative effects [38,39]. According to a recent study conducted by Rothwell et al., the reduced risk of cancer incidence and mortality with aspirin use appeared after 3 years and 5 years, respectively [43]. Although the minimum beneficial duration of aspirin use is not uniform across several studies, the increasing benefits of long-term aspirin use were observed in most of the studies [35,39]. For example, there was a 10% reduced risk of gastric cancer following 4 years of aspirin use (RR = 0.90, 95% CI 0.82–0.99), and this risk reduction is almost doubled for 8-year treatment durations (RR = 0.81, 95% CI 0.67–0.98) and tripled for 12-year durations (RR = 0.72, 95% CI 0.54–0.96) [35]. In addition, the previously observed increased risk of major intestinal bleeding diminished with increasing follow-up time (3 years or greater) [43]. This decrease in risk, coupled with the increasing benefits of long-term aspirin use, suggests that the chemopreventative effects of long-term aspirin treatment may well outweigh the risks over time.

As discussion of the above studies indicates, dose and duration of treatment are two key determinants of the efficacy of aspirin in reducing cancer risk, and although these factors are closely intertwined, they may have separate implications for the potential mechanism of aspirin's action. For example, the dose of aspirin required to exert its effect via COX-dependent mechanisms is relatively lower than through COX-independent mechanisms [44,45], while short-term treatment duration produces effects on post-cancer pathologies and long-term treatment duration produces effects on cancer incidence [46]. Thus, to further elucidate these relationships between the effects of aspirin treatment and its dose and duration, we will discuss the potential mechanisms underlying the anti-tumorigenic powers of aspirin.

4. Mechanistic explanations for the anti-tumorigenic potential of aspirin

Experimental, epidemiological, and clinical data from the last two decades have supported the hypothesis that aspirin possesses anticancer properties. The evidence from this body of research, which spans about 2000 relevant publications, proposes several possible mechanisms of action for the efficacious use of aspirin treatment in multiple cancer types, including colon, breast, prostate, pancreatic, esophageal, leukemic, and lung cancers. Common proposed mechanisms across these studies include induction of apoptosis, anti-proliferative activity, autophagy, and inhibition of angiogenesis and metastasis as the source of the anti-cancer powers of aspirin [46,47].

Aspirin's ability to act on multiple key metabolic and signaling pathways makes it potentially useful in the fight against cancer. As mentioned above, some of these pathways are cyclooxygenase (COX)-dependent, while others are COX-independent [48]. Cyclooxygenases (COXs) are important regulatory enzymes, as they catalyze the rate-limiting step in the production of the prostaglandin H2 from arachidonic acid, which is the precursor of many other prostaglandins and thromboxanes [49]. These regulatory compounds play a role in various biological processes related to cancer, such as cellular proliferation, apoptosis, angiogenesis, metastasis, immune function, and inflammation, all of which are crucial in the development and progression of neoplasms [50]. Aspirin has the capability to inhibit two of these enzymes, COX-1 and COX-2, and, depending on the dose, can produce a dramatic effect in cells such as platelets where COXs cannot be regenerated once they are rendered dysfunctional by aspirin [51].

This mechanism is especially relevant in discussing post-cancer pathologies, where the apparently strong relationship between aspirin use and reduced cancer metastasis is related to this anti-platelet pathway [52]. Low-dose aspirin acts as an anti-platelet agent by causing an irreversible inactivation of platelet COX-1 activity, and thus an inhibition of the synthesis of thromboxane A_2 (TXA_2) [53]. Previous research has established that tumor cells have the ability to aggregate platelets and mask the tumor cells from immune detection, and that platelets thus play a role in the metastatic potential of tumor cells; this association was first reported by Gasic and Gasic in 1968, and is referred to as tumor cell induced platelet aggregation (TCIPA) [54]. TCIPA is an essential immune surveillance escape mechanism for circulating tumor cells, which promotes the survival of circulating tumor cells and facilitates their extravasation and colonization to new microenvironment [55,56]. The relationship between this process and aspirin treatment was further defined in 1972 by a study in which fibrosarcoma cells were injected into the tail vein of mice, and a significant reduction in lung metastasis was observed in the aspirin-treated group [57]. By targeting platelet COXs, aspirin has the potential to prevent platelet-related metastasis through the lymph node [18].

In more recent years, several additional clinical and experimental studies, including a large observational study by Peter Rothwell, further support the promising effect of aspirin on the metastasis of various cancers [27,46,58]. In the course of solid tumor development, angiogenesis is essential for the rapid expansion of a tumor mass [59]. The initiation of this morphogenic process is controlled by the relative balance of pro-angiogenic and anti-angiogenic factors [59]. An increase in the level of TSP-1, an anti-angiogenic protein, while taking aspirin therapy without a concurrent increase in pro-angiogenic protein (such as VEGF) levels results in a tipping of this balance towards anti-angiogenic factors, and thus an inhibition of new blood vessel formation [60]. Under normal physiological conditions, platelets have been shown to release more than 30 pro-angiogenic proteins to promote wound healing. Platelets release more than 80% of the total circulating amount of VEGF, and as such are considered the major source of serum VEGF in both cancer patients and in healthy individuals. In a univariate analysis of women with breast cancer, aspirin use was observed to attenuate this increase in platelet-released VEGF, resulting in an overall decreased serum level of VEGF [61,62]. Additional studies have also presented experimental data showing that overexpression of COX enzymes results in increased serum levels of pro-angiogenic proteins, and that this effect can be reversed through aspirin therapy [63]. This is most reasonably explained by the above hypothesis that aspirin prevents angiogenesis and tumor cell metastasis via inhibition of COX enzymes in the tumor microenvironment and in platelets, as shown in Fig. 1.

Of the two distinct isoforms of the COX enzyme, COX-1 is constitutively expressed in most mammalian tissues and plays an important role in the homeostasis of many physiological processes. The expression of the other isoform, COX-2, is largely induced during inflammation [49], which is a driving force in the development of many tumors; as such, it is widely accepted that alterations in COX-2 expression can influence the development of certain cancers [12,18,64]. For example, COX-2 mRNA has been shown to be overexpressed in nearly 80% of colorectal cancers [65]. This upregulation of COX-2 expression appears to affect colorectal carcinogenesis via a number of distinct mechanisms, such as promoting tumor maintenance and progression, encouraging metastasis, inhibiting apoptosis, and possibly even participating in tumor initiation [50].

It has also been shown that increased COX-2 expression is associated with a concurrent increase in the production of PGE2, a COX-2 derived eicosanoid that modulates cellular proliferation and resists cell death via multiple signaling pathways including MAPK, PI3K, ERK, and cAMP/protein kinase A signaling pathways [66,67]. PGE2 also inhibits programmed cell death by upregulating the expression of the anti-apoptotic protein Bcl-2 in HCA-7, which in turn produces significant amounts of PGE2 to perpetuate this process. PGE2 treatment has also been shown to modulate signaling through the MAPK pathway that leads to upregulation of Bcl-2 [68]. Another key signaling node through which PGE2 acts on apoptosis is the phosphorylation of AKT, which is transactivated by EGFR [69]. As a result of AKT phosphorylation, multiple pro-apoptotic proteins are inactivated, including bad, caspase-9, and forkhead, and multiple anti-apoptotic proteins are activated, including NF-κB and the cAMP response element binding protein [70]. Overexpression of COX-2 derived PGE2 has also been reported to trigger

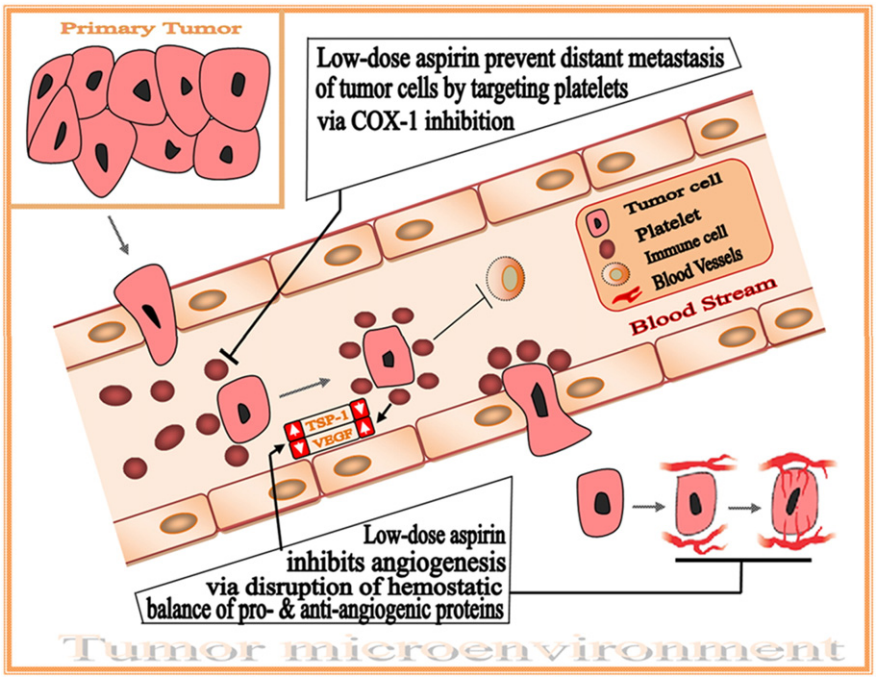

Fig. 1. Aspirin effects mediated via inhibition of COX-1 enzyme. Platelets are involved in the metastasis of cancer by shielding tumor cells in the bloodstream so that they cannot be recognized by the immune system, thus allowing them to ultimately colonize distant organs. Aspirin could help to unmask those tumor cells by targeting platelet aggregation via COX-1 inhibition, allowing immune cells to detect and eliminate them. Platelets are also the major source of angiogenic proteins such as VEGF, and aspirin may disrupt the relative balance of pro- and anti-angiogenic factors released from platelets and prevent angiogenesis, a crucial process in tumor initiation and progression.

GSK3β-, β-catenin-, EGFR-, ERK-, and PI3K-mediated cellular proliferation and differentiation [71,72]. PGE2 inhibition of GSK3β reduces β-catenin phosphorylation and thereby prevents its degradation, leading to the accumulation, nuclear translocation, and functional activity of β-catenin [72]. A recent Nature Medicine study also reported that transactivation of EGFR by PGE2 depends on the extracellular release of an EGF-like ligand, which acts in an autocrine manner [73].

Furthermore, COX-2 upregulation has been shown to be important for Ras-driven transformation. Inhibition of EP receptors blocked growth transformation by Ras, demonstrating that PGE2 upregulation is a key transforming function of COX-2 [74]. Through treating primary mouse keratinocytes with PGE2, this study also demonstrated an increased level of Ras protein, which is secondary to EGFR activation and thus leads to activation of the MAPK and PI3K/AKT pathways. The expression of constitutively active AKT enhanced nuclear peroxisome proliferator-activated receptor-delta (PPARδ) transactivation in the absence of exogenous PGE2, which suggests that PGE2 indirectly enhances PPARδ transactivation via the PI3K/AKT cascade to promote cell survival [75]. Several lines of evidence from recent studies have elucidated the role of aspirin in the induction of apoptosis and inhibition of cell proliferation through its action on the above-mentioned signaling pathways [76–78], a schematic overview of which is provided in Fig. 2. Experimental data also demonstrates that low-dose aspirin is as effective as higher doses in the inhibition of COX-2 activity and decrease of the production of PGE2 in gliomas cells [79]; however, but there is not yet enough evidence to strongly support the hypothesis that aspirin modulates these signaling pathways via its interaction with PGE2. Further research and a clearer understanding of this mechanism might be helpful to predict the efficacy of aspirin treatment in the tumors with constitutively activated signaling pathways due to overexpression of PGE2.

Most often, the dose of aspirin required to block PGE2 synthesis is relatively lower than the dose required for more pronounced anticarcinogenic effects. This observation, when combined with the difference in the clinical activities of low and high-dose aspirin, naturally leads to the hypothesis that not all of the benefits of aspirin are derived from the inhibition of COX, and thus gives rise to the concept of additional mechanisms that are independent of COX activity and prostaglandin synthesis inhibition [44,79]. Multiple recent lines of evidence show that these COX-independent effects of aspirin may be mediated through the inhibition of certain transcription factors, such as NF-κB and AP-1. Aspirin may interfere directly with these transcription factors, but its effects are more probably mediated predominantly through alteration in the activity of cellular kinases, such as IKKβ, Erk, p38 MAPK, or Cdks [45]. One such approach investigated the effect of aspirin on NF-kB signaling and showed its involvement in the inducible expression of a variety of cellular genes that regulate the tissue-specific inflammatory response [80]. The inhibitory protein IkB can sequester NF-kB into cytoplasm, but phosphorylation of IkB by a cellular kinase complex, IKK, leads to degradation and translocation of NF-kB to the nucleus [81]. Once localized in the nucleus, NF-kB binds specific DNA sequences to promote the transcription of genes that influence various physiological processes, including immunity, inflammation, cell proliferation, apoptosis, and even tumorigenesis [82]. Thus, by binding IkB and preventing its phosphorylation by IKK, aspirin has the potential to inhibit the nuclear translocation and subsequent transcription activity of NF-kB [83]. Experimental data also suggests that NF-kB prevent apoptosis by suppression of PTEN, which can be rescued by specific inhibition of NF-kB [84]. PTEN functions as a negative regulator of PI3 kinase/Akt-mediated cell survival pathway, so it may be a potential mechanism by which aspirin used following diagnosis may improve survival in colorectal cancer patients with constitutively activated PI3K pathway signaling when compared to aspirin used prior to diagnosis [12]. Recently, a study concerning the downstream targeting of mTOR effectors revealed that aspirin inhibits the mTOR signaling pathway via activation of AMPK and induces autophagy in colorectal cancer cell lines [76,85].

Aspirin is also of particular interest as a drug that may halt or slow the growth and mutation rate of tumor cells rather than directly killing them. One study conducted in this regard revealed that aspirin decreased the rate of accumulation of genomic abnormalities in the cancerous tissue [86]. These genetic abnormalities accumulate due to defects in homeostatic equilibrium, in which extensive DNA damage is counterbalanced by multiple pathways for DNA repair [87]. One such

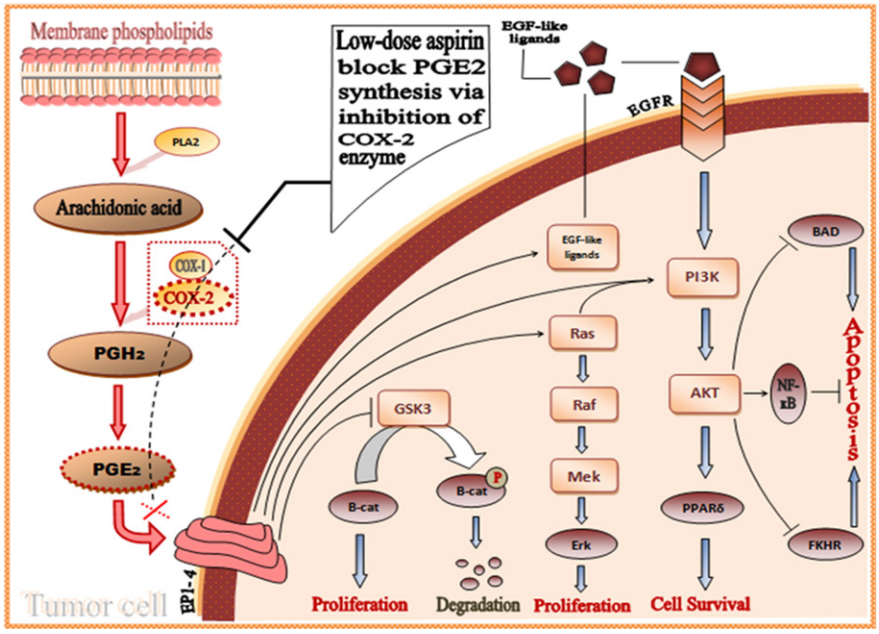

Fig. 2. Aspirin blocks PGE2 synthesis via inhibition of COX-2 enzyme. PGE2 is the major COX-2 metabolite, and thus overexpression of COX-2 stimulates several downstream effectors of PGE2, which in turn modulate cell proliferation and resist cell death. PGE2-mediated inhibition of GSK3β reduces β-catenin phosphorylation and thereby prevents its degradation, leading to its accumulation, nuclear translocation, and functional activity. Transactivation of EGFR by PGE2 also depends on the extracellular release of an EGF-like ligand. Furthermore, PGE2 up-regulation has been shown to activate the Ras–Erk cascade and transduce the signal for cell proliferation. In addition, PGE2 treatment increases the level of Ras protein in mouse keratinocytes, which leads to activation of the PI3K/AKT pathway. Transactivation of the PI3K/AKT signaling pathway by Ras and EGFR then inactivates pro-apoptotic proteins, including bad and forkhead, and activates anti-apoptotic proteins, such as NF-κB. PGE2 also promotes cell survival by transactivation of nuclear PPARδ via the PI3K-AKT cascade. Low-dose aspirin attenuates all of these signaling pathways by inhibiting PGE2 production.

repair pathway, the DNA mismatch repair (MMR) system, is comprised of a complex of proteins, which are regulated in response to mutational damage and can directly repair nucleotide base mismatches or trigger programmed cell death in unfavorable conditions [87,88]. Aspirin is found to increase the steady-state level of MMR proteins in colon cancer cells, thus helping to prevent the accumulation of genetic abnormalities. Furthermore, aspirin induced more profound apoptosis in MMR-proficient cells than in MMR-deficient cells [89], suggesting that aspirin enhances not only the levels of MMR proteins in this DNA repair system, but also their anti-mutagenic performance and may thus exert its cancer-preventing effects by lowering the mutation rate in cells. A brief illustration of these COX-independent mechanisms of aspirin is shown in Fig. 3.

Although hundreds of papers have been published in this field that describe the many different possible modes of actions of aspirin, the exact mechanism by which it interacts with and prevents cancer is not yet fully understood. In this review, we have discussed the most strongly supported mechanisms that have the greatest potential to target cancer cells or to prevent the transformation of normal to cancerous cells. Further research is required to elaborate the exact role of aspirin in cancer prevention, and is a field of considerable ongoing study.

5. Risk-to-benefit ratio of aspirin use in cancer prevention

A growing body of evidence indicates that aspirin can act as a chemopreventative agent, but whether its benefits outweigh its risks is still a developing field. Thus far, clinical guidelines for the prophylactic use of aspirin only consider the cardiovascular benefits of treatment, as these benefits clearly outweigh the potential harm of aspirin-induced gastrointestinal bleeding [90,91]. Such bleeding is the most well-established side effect of aspirin, and a gender-specific study conducted in 2006 demonstrated that aspirin use was significantly associated with an increased risk of a bleeding event both in women (OR, 1.68; 95% CI, 1.13–2.52; $p = 0.01$) and in men (OR, 1.72; 95% CI, 1.35–2.20; $p = 0.001$) [92]. The net impact of aspirin on the risk of upper-gastrointestinal complications (UGIC) for a given patient depends on the presence of a wide range of other gastrointestinal risk factors. For example, the absolute baseline incidence rate for UGIC is 1 in 1000 people per year, a rate which increases exponentially with age from less than 1 in 1000 people per year until the age of 60, to more than 5 per 1000 people per year at age of 85 years. Within each age group, the incidence rate of UGIC among men is double that among women. Another major risk factor is a pre-existing history of a gastrointestinal (GI) ulcer, as the absolute incidence rate of UGIC in patients with or without prior history of a GI ulcer were 1–4 cases and 10–25 cases per 1000 people per year, respectively [93]. Thus, the beneficial effects of aspirin decrease with increasing severity of prior gastrointestinal conditions or other risk factors. However, the use of proton pump inhibitors (PPIs) or *Helicobacter pylori* eradication therapy in combination with aspirin treatment has been shown to be an efficacious approach to circumvent the issue of aspirin-induced GI bleeding [94,95]. Additionally, the evidence for a relationship between aspirin and intracranial bleeding is less clear, and the absolute incidence risk for intracranial bleeding (0.2 in per 1000 patients per year) is much lower than the corresponding risk of GI bleeding. As such, the net benefits of aspirin treatment significantly outweigh the potential harm of intracranial bleeding [96,97].

In consideration of the known risk of bleeding with aspirin treatment, in 2007 the U.S. Preventive Services Task Force (USPSTF) gave aspirin a Grade D recommendation for the prevention of colorectal cancer, meaning its net benefits were only confined to average risk, asymptomatic patients [98]. However, other drug regulatory authorities, such as the American Cancer Society, the National Institutes of Health, and the American Gastroenterological Association, currently do not recommend aspirin use to prevent colorectal cancer [98]. Since 2007, many new lines of evidence have expanded upon the knowledge of the benefits and harms of aspirin for the prevention of several cancers. As we discussed earlier, aspirin has the potential to prevent distant metastasis, angiogenesis, and may also prevent cancer by slowing DNA damage [58,60,86]. These major new benefits have not previously been factored

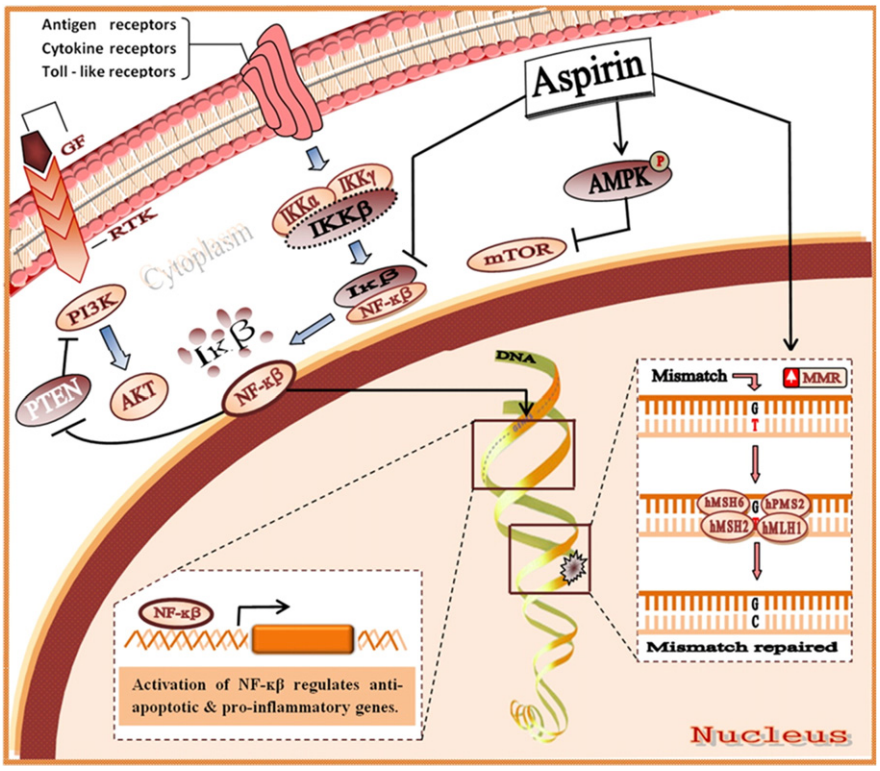

Fig. 3. COX-independent effects of aspirin. High-dose aspirin has the potential to downregulate NF-kB signaling. By binding with IkB, aspirin inhibits the nuclear translocation of NF-kB and prevents its activity in transcription of genes that influence various physiological and pathological processes. In addition, NF-kB also inhibits apoptosis by suppressing PTEN, and this could be rescued by aspirin therapy. Furthermore, inhibition of mTOR signaling as a result of aspirin-induced AMPK activation leads to cellular autophagy. Aspirin also targets and activates the MMR DNA repair system by increasing the steady-state level of MMR proteins, and thus may exert anti-cancer effects by lowering overall mutation rate.

into the recommendations by these drug regulatory agencies, and may prove to tip the balance in favor of aspirin use in cancer prevention.

In 2011, the American Journal of Medicine published a large prospective cohort study for the risk of gastrointestinal bleeding among women enrolled in Nurse's Health Study (NHS). Its results suggest that the risk of gastrointestinal bleeding appears more strongly correlated to dose rather than to duration of aspirin use, and thus the major adverse effect of GI bleeding may be minimized by using the lowest effective dose among both short-term and long-term aspirin treatment [41]. The following year, Rothwell et al. found that low-dose aspirin reduced the risk of the composite outcome of major vascular events, cancer, or fatal extracranial bleeds (HR 0.88, 95% CI 0.82–0.94, $p = 0.0002$), and this benefit remained when non-fatal extracranial bleeds were added as well (HR 0.92, 95% CI 0.86–0.98, $p = 0.01$). Moreover, in contrast with cancer incidence, for which the beneficial effect of aspirin increased with the duration of trial follow-up, its negative effects on major vascular events and extracranial bleeding diminished over time [43]. Additionally, several new recommendations from USA and European clinical guidelines state that PPIs are the preferred agents for the therapy and prophylaxis of aspirin-associated GI injury, which is a positive indication of the safe use of aspirin [99,100].

Based on these recent developments, we suggest that future clinical guidelines for the prophylactic use of aspirin also consider the chemopreventive effects as well as the cardiovascular benefits of aspirin treatment. Although the risks of aspirin treatment cannot be ignored, updated recommendations and guidelines for the use of aspirin are appropriate considering the disease burden of cancer and aspirin's great potential in its prevention.

6. An emerging clinical entity: “aspirin resistance”

Fluctuations in the efficacy of aspirin therapy give rise to the concept of aspirin resistance. This term is widely studied in the cardiovascular context of aspirin use, and although there is no clearly defined condition, a vast majority of studies have reported that the occurrence of aspirin resistance in different clinical settings has relied on ex vivo measurement of platelet function. This measurement faithfully represents the ability of aspirin to inhibit COX-1-induced platelet aggregation [101,102] and may thus be an accurate prognostic marker for aspirin resistance in the clinic. Several underlying mechanisms for aspirin resistance have been proposed, and are discussed elsewhere in detail [103,104].

The term “aspirin resistance” may not be relevant in the context of cancer prevention, as it has been widely established in the field that the chemopreventive effects of aspirin were not only achieved by platelet inhibition, but also through several other mechanisms that contribute to its overall effect [78]. Thus, we can hypothesize that only one portion of aspirin's benefit is lost when resistance results in its failure to block COX-1-induced platelet aggregation. However, recent studies have linked the efficacy of aspirin treatment in cancer prevention to the individual genetic landscape of the tumors [12,105]. For example, BRAF-mutant colon tumor cells were less sensitive to the effects of aspirin when compared with BRAF-wild type colon tumor cells [105], suggesting that the mutational profile of the tumor may be one of the possible factors that results in the observed variance in response to aspirin therapy.

7. Conclusion

Despite the challenges and complex nature of the use of aspirin in cancer prevention, it remains an intriguing and promising field of research. We have discussed here the many potential beneficial effects of aspirin treatment in not only reducing cancer risk, but also in preventing metastasis and cancer-associated mortality. We have also addressed many possible mechanisms through which aspirin use may exert these beneficial effects, including both COX-dependent and

independent mechanisms. Finally, we have reviewed the potential risks of aspirin use and the balance of these risks with the many benefits. In the future, the use of animal models in this setting may provide data that better reflects clinical responses. Of particular utility in this context are patient-derived xenograft (PDX) models, which are currently used to evaluate novel targeted therapeutic strategies and their effects on metastatic dissemination and tumor growth. In addition to this current use, PDX models may also elucidate the basis for sensitivity or resistance of tumors from individual patients to aspirin use. Furthermore, future clinical trials formally addressing the role of aspirin therapy will need rigorous attention to patient selection, combination therapy with existing agents, and trial endpoints, which all provide significant opportunities to improve cancer outcomes. The use of aspirin in cancer prevention is clearly a rapidly developing field, and many crucial developments in our understanding of its use in this context remain yet to unfold.

Transparency document

Transparency document associated with this article can be found, in the online version.

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