Anti-tumor Effects of Aspirin: Progress in Clinical and Basic Studies

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Abstract: Beyond the antipyretic analgesic and anti-inflammatory effects for which aspirin has historically been used, studies have shown that aspirin also plays an important role in the prevention or treatment a variety of diseases. The anti-tumor effects of aspirin have received increasing attention during the past decade. Many studies have explored the molecular mechanisms underlying these anti-tumor effects *in vitro* and *in vivo*, and abundant discoveries have been made through observational or interventional clinical studies. In terms of its molecular function, aspirin has been shown to prevent tumor cell growth through inhibiting the signal transduction of the COX, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), NFκB/IκB, and Bcl-2/Bax pathways. Under certain conditions, aspirin can also induce autophagy, which is an inhibitory mechanism for some tumors. This article provides a comprehensive overview of the anti-tumor effects of aspirin and discusses the concrete mechanisms underlying aspirin's anti-tumor effects that have been discovered in the past 30 years.

Key words: Aspirin; Anti-tumor; Clinical research; Molecular mechanism; Non-steroidal anti-inflammatory drugs

Introduction

Aspirin has long been known to have activity in a variety of diseases, ranging from antipyretic, analgesic and anti-inflammatory effects to utility in treating cardiovascular disease [1]. However, the more recent discovery of anti-tumor effects has led to renewed interest in aspirin, resulting in both clinical studies and basic research. The anti-tumor effects of aspirin were first discovered in colorectal cancer [2]. Subsequent studies have found that the anti-tumor effects extend to various other malignancies, such as gastric [3], breast [4], ovarian [5], liver [6], prostate [7], esophageal [8] and other cancers [5,7-11]. Randomized and observational trials were conducted via meta-analyses, and showed that aspirin can reduce the incidence of multiple adenocarcinomas [3,12,13]. This paper summarizes the recently conducted clinical studies and basic experiments, and explains the results and their implications.

Clinical Studies on the Anti-tumor Effects of Aspirin

Aspirin was first found to have anti-colorectal cancer effects about two decades ago [14]. As early as 1998, there were data suggesting that aspirin could reduce the risk of colon cancer, but the initial study was largely overlooked because of the small sample size [15]. Subsequently, other randomized trials showed that aspirin reduced the risk of colorectal neoplasia [16,17]. Rothwell PM et al. analyzed four randomized trials of aspirin versus control (used in the primary and secondary prevention of vascular events) and found that aspirin reduced both the 20-year risk of colon cancer (HR: 0.76,

0.60-0.96, $P = 0.02$) and colon cancer-related mortality (HR: 0.65, 0.48-0.88, $P = 0.005$), but did not affect rectal cancer [14]. In 2013, a larger trial including 5,071 women proved that aspirin could reduce the risk of colon cancer in women [18]. Since then, numerous studies have demonstrated the anti-tumor effects of aspirin in gastric [3], breast [4], ovarian [5], liver [6] and prostate [7] cancer. It is now well-accepted that aspirin has inhibitory effects against a variety of tumors. Following these discoveries, researchers began to explore the impact of the frequency and dosage of aspirin treatment.

The Optimal Duration of Treatment

Many studies have confirmed that the long-term use of aspirin has clear anti-tumor effects. Rothwell PM et al. [19] investigated the anti-tumor effects of aspirin by screening out two distinct groups of patients (aspirin-users and aspirin-free) with an average treatment time of more than 4 years. From the eight eligible studies they evaluated, it was clear that aspirin had anti-tumor effects that were independent of the treatment dose (more/less than 75 mg), and aspirin treatment reduced the mortality risk of many cancers when oral aspirin use was sustained for at least five years [13]. Aspirin reduces the development of esophageal cancer [8], pancreatic cancer, gastric cancer, colorectal cancer and prostate cancer [5], but their prevention seems to require sustained use of aspirin for at least 5 years, with the anti-tumors effects becoming more prominent with an increasing duration of aspirin use [13]. Aspirin did not have any apparent effect when used for less than 5 years, and required even longer for any effect on gastric, colorectal and prostate cancer. Based on these studies, the consensus of researchers is that patients need to take oral aspirin regularly for at least 5 years to experience anti-tumor effects.

The Impact of Dose

High-dose aspirin (≥ 500 mg daily) aspirin reduces the long-term incidence of colorectal cancer, but adverse effects (particularly bleeding) might limit its potential for long-term prevention [14]. In Sandler RS's randomized trial, taking 350 mg of aspirin per day prevented the development of colorectal adenomas in patients with previous colorectal cancer, and the adjusted relative risk of any recurrent adenoma in the aspirin group, compared with the placebo group, was 0.65 (95 % CI: 0.46-0.91) [16]. Rothwell PM et al. also conducted a study to investigate the morbidity and long-term bleeding risk of the aspirin and aspirin-free groups [20]. They concluded that taking low-dose aspirin (at least 75 mg everyday) reduced the risk of death due to cancer, as well as the risk of intracranial hemorrhage. Two large-scale randomized trials conducted by the Women's Health Study [5] and the Physicians' Health Study showed no association between taking aspirin every other day and the risk of developing colorectal cancer [18].

Although Rothwell PM's meta-analysis suggested that aspirin had anti-cancer effects regardless of the dose taken [19], Clarke CA et al. found that developing hormone receptor-positive/HER2-negative breast cancer was inversely associated with taking three or more tablets of low-dose aspirin (100 mg) per week. [4]. A retrospective study of 4,627 women with breast cancer showed that low-dose aspirin (75 mg/day) was associated with a decreased risk of breast cancer-related death [21]. Trabert B et al. found that taking aspirin was associated with a lower risk of ovarian cancer, especially when low-dose aspirin (< 100 mg) was taken daily [22]. Gray RT et al. similarly found that the daily use of low-dose aspirin (75 mg) was associated with a 30% reduction in the risk of colorectal cancer [23]. Cook NR et al. performed a randomized controlled trial, which showed that the long-term use of low-dose aspirin reduced the risk of CRC in healthy women [18]. However, Mathers JC et al. showed that if patients with hereditary CRC took an average of 600 mg of aspirin per day for 25 months, their incidence of rectal cancer was halved, so higher doses may be needed to achieve an effect in some patients [2].

The Anti-Tumor Effects Apply to Numerous Cancer Types

As noted above, aspirin has effects on a variety of

tumors. A prospective study of 4,164 women with breast cancer showed that the use of aspirin reduced the risk of breast cancer recurrence and death [4]. A retrospective study of 148,739 women with diabetes showed that using aspirin reduced the risk of breast cancer by 18% [21]. Aspirin and anti-angiogenic therapy showed synergistic effects against human glioblastoma multiforme (GBM) [24]. In addition, the same aspirin regimen reduced the risk of ovarian cancer by 20% to 34%, depending on the frequency of use [24]. Rothwell PM et al. [20] analyzed the individual patient data from 7 trials (23,535 patients with all cancers, 657 cancer deaths) after 5 years of follow-up, and found that the risk of cancer death in the aspirin group was lower than that of the control group, with particular benefit to patients with cancers of the gastrointestinal tract. The benefits to patients with lung cancer and esophageal cancer were limited to adenocarcinoma, and aspirin had the greatest overall impact on the risk of adenocarcinoma-related death (HR = 0.66, 95%CI = 0.56-0.77, $P < 0.0001$) (Table 1).

The Risks Associated with Aspirin and Recommendations on Preventive Dosing

Higher doses of aspirin may cause peptic ulcers and a bleeding tendency, and some patients may develop a rash or urticaria. Godley RW et al. [1] found that for patients with known cardiovascular disease (CVD), taking aspirin reduced the risk of cardiovascular events, and these benefits outweighed the risk of bleeding. The American College of Cardiology/American Heart Association (ACC/AHA) recommended that in the absence of contraindications, 75 mg-160 mg of aspirin should be used by patients with CVD. The 2016 United States Preventive Services Task Force (USPSTF) released the latest recommendations for aspirin for CVD and CRC. For high-risk populations between the ages of 50 and 59 with CVD and CRC, it is recommended that all patients take low-dose aspirin daily for at least 10 years [25]. Although the evidence is relatively weak, the benefits are thought to outweigh the potential risks. This advice is also given to people at risk of CVD and/or CRC, or who are under the age of 70 [25].

Confounding Factors

According to a recent study by Gray RT et al., the use of low-dose aspirin does not prolong the survival of patients with colorectal cancer [23]. Hannibal CG et al. reported

Table 1 The relationship between the antitumor effects of aspirin and the dose taken

Authors	Dose of Aspirin	Research finding
Rothwell PM et al [19]	> 75 mg/day, > 5 years	Reduce the incidence of esophageal cancer, pancreatic cancer, lung cancer, gastric cancer, colorectal cancer and prostate cancer
Clarke CA et al [4]	100 mg, at least 3 times/week	Reduced the risk of hormone receptor-positive/HER2-negative breast cancer
Leite AM et al [21]	75 mg/day	The risk of colorectal cancer was 30% lower
Mathers JC et al [2]	600 mg/day, > 25 months	The incidence of rectal cancer decreased by 50%
Trabert B et al [22]	> 75 mg/day, > 5 years	The risk of ovarian cancer was decreased 20% to 34%

that low-dose aspirin did not prevent ovarian borderline tumors, but larger studies may be required to find potential links between aspirin and ovarian borderline tumors [26]. Because healthy individuals may be unwilling to take aspirin every day, it may be more appropriate to develop a treatment program for individuals with existing colorectal lesions.

In general, recent studies have confirmed the anti-tumor effects of aspirin for a variety of cancer types, but the research suggests that the dose should be higher than 75 mg/day, and it should be taken regularly for at least 5 years.

Mechanisms Underlying the Anti-tumor Effects of Aspirin

Numerous studies have been performed to explore the specific mechanism(s) underlying the anti-tumor effects of aspirin. It has been suggested that the anti-tumor activity of aspirin mainly involves its effects on the cyclooxygenase (COX) pathways, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), transcription factors AP-1 and NFκB, Bcl-2, oxidation, stress and autophagy.

COX pathways

There are two cyclooxygenases (prostaglandin-endoperoxide synthase) isoenzymes: COX-1 and COX-2. COX-1 is a structural type enzyme that exists in normal tissues, where its specific functions are related to regulating gastrointestinal mucus secretion, platelet aggregation, vascular tone and the renal blood flow distribution after inflammation. COX-1 is involved in the synthesis of thromboxane α_2 (TXA₂), and therefore plays an important role in platelet aggregation [6]. A variety of studies have shown that tumor cells use platelet aggregation to escape immune surveillance and migrate to new locations [27,28]. Therefore, it has been speculated that platelets play an important role in tumor metastasis and dissemination via a phenomenon called tumor cell-induced platelet aggregation (TCIPA) [29]. Aspirin can inhibit the release of platelets by COX-1, causing platelet dysfunction, and can also induce irreversible inactivation of COX-1, indirectly affecting platelet aggregation, and thus tumor cell dissemination and metastasis.

In addition, the formation of blood vessels plays a decisive role in the proliferation of tumor cells by providing the necessary nutrients for tumor cell growth and division, as well as dissemination to new sites. Therefore, pro-angiogenic factors and angiogenic factors are critical for the formation of primary and metastatic tumors. Under normal physiological conditions, platelets release more than 30 angiogenic factors that promote wound healing [30], however vascular endothelial growth factor (VEGF) accounts for more than 80% of the angiogenic factors released by platelets [31]. An increase in platelets in cancer patients leads to enhanced secretion of VEGF, which nourishes vascular proliferation around tumor cells

and provides nutrients for tumor cells and a pathway for dissemination. Aspirin can inhibit the overexpression of platelets and decrease the level of VEGF in the serum. When the level of VEGF decreases, it reduces angiogenesis. Simultaneously, the transport of nutrients to tumor cells is reduced, inhibiting the growth and proliferation of tumor cells [15,32].

COX-2 is an inducible enzyme that exists in the central nervous system (CNS), kidneys and the vascular endothelium. Its specific function is to produce inflammatory substances. In human bone marrow cells and cancer cells, the enzyme is also involved in the conversion of arachidonic acid to prostaglandin E₂ (PGE₂) [33,34]. In a study of lung cancer cells, PGE₂ was found to promote the metabolic proliferation of tumor cells through various signaling pathways, such as the mitogen-activated protein kinase (MAPK), P12K, ERK, cAMP/PK and other signaling pathways, and also inhibited cell apoptosis. Inhibiting the COX-2 pathway decreases tumor cell proliferation. In colorectal and breast cancer cells, aspirin also accelerated cell death by inhibiting the expression of COX-2 and blocking the phosphatidylinositol 3-kinase-related pathway (PIK3CA) [35]. A summary of these mechanisms is shown in Figure 1.

Figure 1 The molecular mechanisms underlying how aspirin exerts anti-cancer effects via the Cox pathways

TRAIL Pathway

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a trimetric protein that activates the TRAIL receptor on the cell membrane to induce apoptosis [36]. Previous studies demonstrated that TRAIL can selectively kill tumor cells [37], and endogenous TRAIL plays an important role in inhibiting cancer, as TRAIL deficiency accelerates the growth of mouse malignancies [38]. There

are five receptors for TRAIL: DR4 (TRAIL-r1), DR5 (TRAIL-r2), DcR1, DcR2, and OPG. Only DR4 and DR5 can transduce apoptosis signals [39]. TRAIL induces death signal complex assembly by binding to DR4 and DR5, triggering caspase-dependent apoptosis [40]. DR5 is more active at normal human body temperature, so under normal circumstances, DR5 is the main receptor activated to kill tumor cells in the human body. In normal cells, DR4 and DR5 are barely expressed, while the receptors are expressed on several types of cancer cells [40]. Insufficient expression of DR4 and DR5 on the surface of tumor cells was shown to be closely related to the resistance of breast cancer cell lines to TRAIL [41].

Autophagy is a recently-discovered mechanism of cell death associated with the dynamic regulation of TRAIL death receptors. It is a natural cellular catabolic process in which cytoplasmic components are sequestered in island autophagosomes and then degraded by lysosomal pathways. Autophagy can also be induced by breast cancer cells to reduce the expression of DR4 and DR5 on the cell surface and induce TRAIL resistance. Restoration of DR4 and DR5 surface expression can be achieved by inhibiting or disrupting protein transport, endocytosis, or autophagy [39]. Aspirin can sensitize cells to TRAIL-induced apoptosis [40]. Although the exact mechanism is unclear, recent studies suggest that aspirin can enhance the expression of DR5 receptor in human breast cancer cell lines and xenografts [40]. Aspirin promotes caspase activation by TRAIL, activates caspase-dependent Mcl1 cleavage, and triggers the mitochondrial apoptotic pathway, thereby inducing tumor cell death [41].

NF- κ B/I κ B Pathway

The anti-tumor activity of aspirin also appears to involve the activation of transcription factor activator protein 1 (AP-1) and nuclear factor κ B (NF- κ B). AP-1 regulatory genes play various biological functions in tumor cells, including roles in cell proliferation and differentiation, apoptosis, transformation, invasion, and metastasis [42]. NF- κ B is an important transcription factor that regulates the malignant phenotype of cancer gene programming. Activation of NF- κ B can promote the expression of COX-2 and alter the activity of certain cell kinases, such as I κ B kinase (IKK), extracellular signal-regulated kinase (ERK), and cell cycle-dependent protein kinases (CDKs) [43]. I κ B (inhibitor of NF- κ B) maintains NF- κ B in the cytoplasm. When I κ B is phosphorylated by IKK, it separates from NF- κ B, allowing it to enter the nucleus and bind to specific DNA sequences to promote gene transcription. These genes are involved in a variety of physiological processes, including immunity, inflammation, cell proliferation, apoptosis, and tumorigenesis [44].

Aspirin inhibits NF- κ B in several ways. First, aspirin binds to IKK, thereby inhibiting I κ B phosphorylation.

Second, aspirin inhibits IKK-2 activity by binding to IKK-2, thereby reducing Akt-2 and ATP. Aspirin can therefore both inhibit the entry of NF- κ B into the nucleus and prevent a series of post-translocation activities, thereby inhibiting the growth of tumor cells [45,46]. In addition, NF- κ B has been shown to inhibit PTEN (a tumor suppressor gene) gene activity. The PTEN protein is a negative regulatory protein that is a key part of the PI3k/Akt pathway. The PI3k/Akt pathway regulates cell proliferation and apoptosis and increases the expression of inducible nitric oxide synthase (iNOS) and COX-2 in inflammatory cells. NF- κ B enhances the PI3 kinase/Akt-mediated cell survival pathway and inhibits apoptosis by inhibiting PTEN. Thus, aspirin may reduce this cyclic loop of activation. In summary, aspirin inhibits the nuclear translocation of NF κ B in human cancer cells and promotes apoptosis in tumor cells via several mechanisms [47,48].

Bcl-2 and Bax Pathways

There are two main apoptotic signaling pathways: the exogenous pathway and the endogenous pathway. In the exogenous pathway, the binding of an external ligand to a receptor causes the direct activation of caspases, which induce apoptosis. In the endogenous pathway, the “death ligand” originates inside of the cell, for example, via activation of p53 after DNA damage, with apoptosis first involving the mitochondria.

The B-cell lymphoma/leukemia-2 (Bcl-2) family is a group of highly conserved proteins containing or more of four homologous domains, termed BH-1, BH-2, BH-3, and BH-4. Some family members are anti-apoptotic proteins (e.g., Bcl-2 and Bcl-xL), some are pro-apoptotic proteins (Bax, Bak and Bok) and some have only BH3 domains (e.g., Bid, Bad, Puma, and Bim). The Bcl-2 gene encodes a mitochondrial protein that enhances the mitochondrial membrane potential and reduces calcium ion transport across the mitochondrial membrane, leading to inactivation of endonucleases, thereby inhibiting cell death [49]. Activation of the cellular signaling pathway associated with Bax promotes apoptosis by activating caspase-specific proteases and altering the cell membrane permeability [50,51]. The ratio of Bax to Bcl-2 is an important factor that regulates tumor cell apoptosis. When the Bax content is higher than the Bcl-2 content, the formation of homodimeric Bax-Bax promotes tumor cell apoptosis; when the Bax protein content is less than the Bcl-2 content, the formation of heterodimeric Bax-Bcl-2 increases, leading to inhibited apoptosis. In hepatocellular carcinoma HepG2 cells, there was an increase in Bax and a slight decrease in Bcl-2 expression after aspirin treatment [52]. This data suggests that aspirin treatment alters the proportion of Bax/Bcl-2 in HepG2 cells, and this may induce apoptosis [52]. In addition, studies have shown that in multiple myeloma cells and xenograft models, aspirin can inhibit tumor cell proliferation and induce apoptosis by

up-regulating Bax, down-regulating Bcl-2, and changing the Bax/Bcl-2 ratio [52].

Aspirin May Alter Autophagy and Other Cellular Processes

Autophagy occurs frequently during tumorigenesis and cancer chemotherapy, and is considered to be a target for cancer therapy [53]. As noted above, autophagy has been linked to the TRAIL pathway. Breast cancer cells can reduce their sensitivity to TRAIL through the selective induction of autophagy. Studies have shown that aspirin can reduce this phenomenon, but the underlying mechanism is unclear. In addition, in mouse liver cancer and sarcoma models, aspirin inhibited tumor angiogenesis by inhibiting autophagy via inhibition of the mTOR signaling pathway [54]. However, combination therapy using aspirin plus ABT-737 (a Bcl-2 inhibitor) induced greater autophagy than ABT-737 used alone [55]. Aspirin was effective at reducing the mean total number of adenomas per participant, and the number of adenomas in the right-sided colon [56]. Therefore, the impact of aspirin on autophagy remains unclear, and may be cancer-specific or related to other factors.

In addition to the various studies described above, it was recently demonstrated that aspirin can be encapsulated in tumor-targeting vesicles, and these could induce anti-proliferative effects against epidermoid carcinoma, melanoma and glioblastoma [57]. Another study showed that aspirin can inhibit cancer growth and dissemination by affecting the tumor microenvironment [58]. Thus, there are a wide variety of possible targets and mechanisms of action underlying the anti-cancer effects of aspirin.

Conclusion

In summary, although the anti-tumor effects of aspirin have been well-documented, the underlying mechanisms are still being elucidated. In addition, further research is needed to clarify the optimal dosing and duration of treatment in order to prevent or treat various human malignancies.

Abbreviation

AP-1, activator protein 1
Bcl-2, B-cell lymphoma-2
Bax, Bcl-2-associated X
CDKs, cell cycle-dependent protein kinases
COX1, cyclo-oxygenase1
COX2, cyclo-oxygenase2
CRC, colorectal cancer
CVD, cardiovascular disease
CNS, central nervous system
CDKs, cyclin-dependent protein kinases
DR, death receptor
ERK, extracellular signal regulated kinase
GBM, glioblastoma multiforme
HER-2, human epidermal growth factor receptor-2
IkB, inhibitor of NFκB

IKK, IkB kinase

iNOS, inducible nitric oxide synthase

MAPK, mitogen-activated protein kinase

NFκB, nuclear factor kappa-B

PGE2, prostaglandin E2

TXA2, thromboxane A2

TCIPA, tumor cell-induced platelet aggregation

USPSTF, United States Preventive Services Task Force

VEGF, vascular endothelial growth factor

Conflict of interests

The authors report no conflicts of interest related to this work.

Funding

This work was supported by the National Key Clinical Specialist Construction Project Funding Project (No. 09507), Shanxi Province Health Commission Scientific Research Projects (No. 2018037) and Shanxi Province Key Research Social Development Project (No. 201703D321010-2).

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