

# Mechanisms of the Antiangiogenic Effects for Cancer by the Aspirin

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## Abstract

Aspirin as an old drug extracted from willow bark and widely used in the prevention and treatment of cardiovascular diseases. Increasing evidence have shown that aspirin use may significantly reduce the angiogenesis of cancer, while the mechanism of the association between angiogenesis and aspirin is complex. Although COX-1 is widely known as a target of aspirin, several studies reveal other antiangiogenic targets of aspirin, such as angiotensin II, Glucose transporter 1, heparanase, and matrix metalloproteinase. In addition, some evidence indicates that aspirin may produce antiangiogenic effects after acting in different cell types, such as endothelial cell, platelet, pericyte, and macrophage. In this review, we concentrate on recent researches on the antiangiogenic effects of aspirin in cancer, and analyze the molecular mechanisms of aspirin and its metabolites. Moreover, we discuss some mechanisms through which aspirin treatment may normalise existing blood vessels, including preventing disintegration of endothelial adheren junctions and recruiting pericytes. We also address the antiangiogenic effects and the underlying mechanisms of aspirin derivatives, which aim to improve safety and efficacy.

## 1. Introduction

Angiogenesis is the process through which new capillaries are sprouted from pre-existing ones (Eilken et al., 2010). And the components of the vascular wall are basement membrane, pericytes, vascular smooth muscle cells and endothelial cells (Carmeliet et al., 2011). The concept of antiangiogenic therapy, inhibition of angiogenesis could inhibit tumor growth, started after an observation by Folkman discovering new vessel production during tumors growth (Folkman, 1971). It is now relatively cleared that angiogenesis plays an important role in tumor metastasis and growth (Vasudev et al., 2014; Bielenberg et al., 2015). The angiogenic system is required for tumor tissue to grow and develop, and angiogenesis is a complex process involving an interaction between soluble factors, extracellular matrix (ECM) components and endothelial cells(ECs) (Patan, 2000). The early stage of angiogenesis occurs dynamic functions of ECs including proliferation, migration and maturation (Griffioen et al., 2000). The significant event in tumor angiogenesis is endothelial cell migration, which relays on the assembly of protease-protein complexes in front of migrating cells to degrade the basement membrane and invade the matrix of adjacent tissues (Ausprunk et al., 1977; Liekens et al., 2001). Notably, the chronic imbalance of proangiogenic and antiangiogenic factors leads to abnormal angiogenesis in tumor tissues (Goel et al., 2011; Carmeliet et al., 2011).

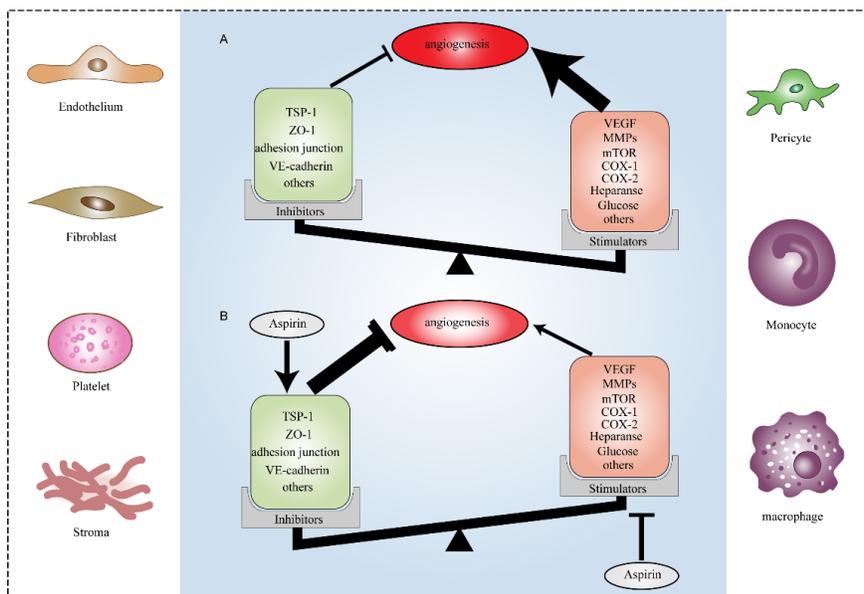
Aspirin (acetylsalicylic acid, ASA) is an old medicine that was originally extracted from willow bark and is widely used in the prevention and treatment of cardiovascular and cerebrovascular diseases (Desborough et al., 2017). It is absorbed in the gastrointestinal tract after oral administration of aspirin in man, and then converted into salicylic acid (SA) which is further metabolized to salicylic acid, gentisic acid, gentisuric acid (2,5-DHBA), salicyl acyl glucuronide, and salicyl phenolic glucuronide (Rumble et al., 1981). During the circulation process, the biological activity of salicylic acid is impaired due to the large amount of salicylic acid (50-80%) bound to albumin, which is because the free salicylic acid has biological activity (Hua et al., 2019).

Increasing evidence have shown that long-term regular aspirin use can significantly reduce the risk of overall

cancer (Cao et al., 2016). These effects may be related to the antiangiogenic ability of aspirin (Pearce et al., 2003; Yoshida et al., 2003; Tsujii et al., 1998). Here, we update recent researches on the antiangiogenic effects of aspirin in cancer prevention and treatment, and analyze the several molecular mechanisms of the antiangiogenesis effects for cancer by aspirin.

## 2. Antiangiogenic mechanisms for the prevention and therapy of cancer by aspirin

In angiogenesis, the therapeutic concentration of aspirin can cause a significantly reduction in vessel tubule formation, while the high levels of endothelial cell apoptosis could triggered by aspirin at the higher concentration (Borthwick et al., 2009). Moreover, present studies show that aspirin significantly inhibits proliferation of normal human dermal microvascular endothelial cells (HuDMEC) and human umbilical vein endothelial cells (HUVEC) (Pearce et al., 2003; Boonmasawai et al., 2009). Furthermore, ASA also blocks endothelial cell migration after stimulation with VEGF (Navone et al., 2018), cancer cell (Maity et al., 2019) or ADP (Battinelli et al., 2011). However, the mechanism of these effects is complex. Many endogenous angiogenesis stimulators and inhibitors reside within the host microenvironment, including Interleukin-8 (IL-8), Fibroblast growth factor-1 (acidic FGF, FGF1), Fibroblast growth factor-2 (basic FGF, FGF2), Vascular endothelial growth factor (VEGF), vascular permeability factor (VPF) and Platelet-derived growth factor (PDGF) as well as others (Li et al., 2012). Aspirin could suppress angiogenesis and curb tumor growth by inducing these inhibitors and inhibiting these stimulants (Figure 1).



**Figure 1** The role of aspirin to tumor angiogenesis inhibition by regulation a balance of endogenous inhibitors and stimulators. (A) In the tumor microenvironment, the sum effect of these inhibitors and stimulants is angiogenesis with abnormal structure and function. This imbalance proangiogenic effects is aggravated by activated platelets, monocytes and macrophages. (B) Aspirin may reverse the balance of antiangiogenic and proangiogenic effect in angiogenesis through lower endogenous stimulators and boost inhibitors. Reverse of antiangiogenic and proangiogenic balance may inhibit angiogenesis, normalize existing blood vessels and reduce permeability and leakiness.

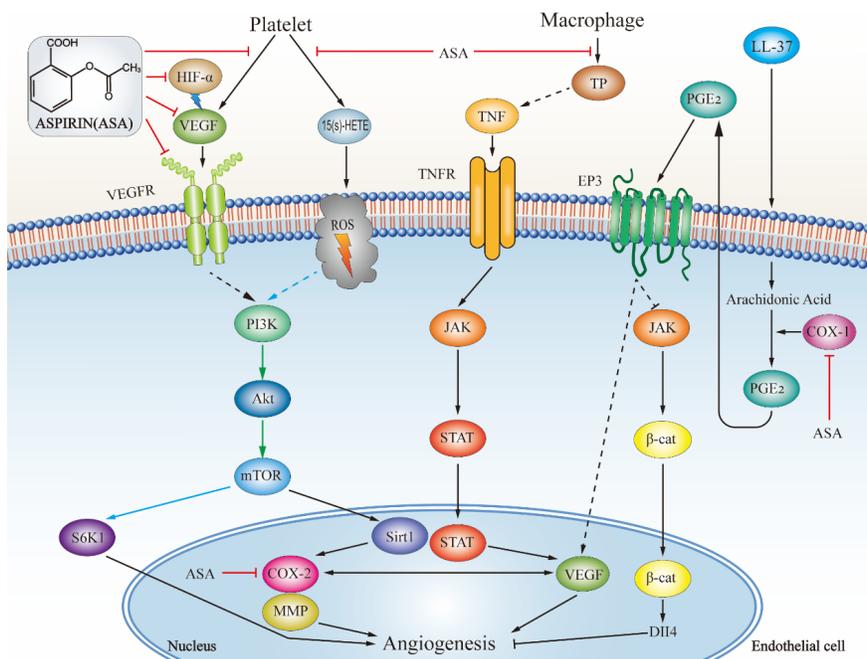
Abbreviations: TSP-1, thrombospondin-1; ZO-1, Zonula Occludens-1; VEGF, Vascular endothelial growth factor; FGF, Fibroblast growth factor; MMPs, matrix metalloproteinases; mTOR, Mammalian target of rapamycin.

### 2.1 Effect on Heparanase and the matrix metalloproteinases

Heparanase, the heparan sulfate (HS)-degrading endo- $\beta$ -D-glucuronidase, could regulate angiogenesis and degrade the HS chain of heparan sulfate proteoglycans in the extracellular matrix (Li et al., 2009; Vlodaysky et al., 2018). After the extracellular matrix collapses, heparin-binding cytokines such as HGF, VEGF, and bFGF are released to promote tumor angiogenesis (Talmadge et al., 2010; Vlodaysky et al., 2001). Notably, except for the members of FGF-19 subfamily with little or no affinity for these glycosaminoglycans, all fibroblast growth factors high affinity bind to heparin (Asada et al., 2009). Heparanase is inhibited by aspirin which directly bind to Glu150 (human Q9Y251: Glu 225) region and thereby suppress the vascular structures formation in a dose-dependent manner (Dai et al., 2017). Salicylic acid also has the same effect of inhibiting the heparanase activity (Dai et al., 2017). Therefore, aspirin or salicylic acid may reduce release of heparin-binding cytokines such as HGF, VEGF, and bFGF, thereby inhibiting angiogenesis.

The matrix metalloproteinases (MMPs), a large family of zinc-dependent endoproteases, can remodel and degrade the components in the extracellular matrix (ECM) and could play a vital role in angiogenesis (Kapoor et al., 2016). The degradation effect of Gelatinase-B (MMP-9) in the extracellular matrix promotes the migration of ECs to angiogenesis stimuli (Hiraoka et al., 1998; Puyraimond et al., 1999). Moreover, MMP-9 can degrade the Multimerin 2, which is tightly juxtaposed with ECs surface and bind proangiogenic cytokines to exert angiostatic functions (Andreuzzi et al., 2017; Christian et al., 2001). Metalloproteinase 1 (TIMP-1) as a specific tissue inhibitor could inhibit MMP-9 and suppress the ECM degradation in tumor angiogenesis (Khokha et al., 1994). In addition, it has been demonstrated that MMP-9 can induce the expression of VEGF (Bergers et al., 2000). Interesting, aspirin can suppress the levels of secreted MMP-9 activity and increase the levels of secreted TIMP-1 activity in the cancer cell (Shi et al., 2017). In this way, aspirin may inhibit migration of ECs to angiogenesis stimuli in the tumor microenvironment. Furthermore, EP3 receptor signal transduction on ECs is crucial for MMP-9 upregulation, which can enhance tumor angiogenesis (Amano et al., 2008). It has been demonstrated that aspirin can reduce MMP-9 upregulation by inhibiting cPLA2-COX-1-PGE2-EP3 Signaling in ECs (Salvado et al., 2013).

## 2.2 Effect on Cyclooxygenase of endothelial cells



The expression and activity of COX-1 in HUVEC regulates the formation of tubular structures induced by cancer cells (Tsuji et al., 1998). LL-37, the only cathelicidin protein expressed by humans, upregulated in

breast, ovarian and lung tumors (Larrick et al., 1995; Coffelt et al., 2009; Weber et al., 2009; Haussen et al., 2008). The Cathelicidin LL-37 can induce a proangiogenic response via cPLA2-COX-1-PGE2-EP3 Signaling in human ECs, which is suppressed by aspirin in a manner of inhibiting COX-1 in vivo (Salvado et al., 2013) (Figure 2). Activation of PGE2/EP3 axis promotes sprouting angiogenesis by suppression of protein kinase A (PKA)/p-S675-  $\beta$ -catenin/Notch signaling in ECs (Chen et al., 2017). Moreover, fibroblast growth factor-1 and fibroblast growth factor-2 are the first two discovered polypeptides showing proangiogenic effects (Thomas et al., 1985; Esch et al., 1985). FGFR1 (Fibroblast Growth Factor Receptor 1) pathway in microvascular endothelial cells can be activated by EP3 receptor signal mediation (Federica et al., 2008). The 2,5-dihydroxyphenylic acids (Gentisic Acid), a metabolite of Aspirin, is a powerful fibroblast growth factor Inhibitor (Grootveld et al., 1998; Fernández et al., 2010). Furthermore, angiogenic effects of PGE2 are mediated by up-regulation of the C-X-C chemokine receptor type 4 (CXCR4) and CXCR4 expression can be inhibited by aspirin in HMECs (Rosalba et al., 2003). However, it warrants further research to verify the downstream of cPLA2-COX-1-PGE2-EP3 signalling transduction when aspirin antiangiogenesis.

**Figure 2 Shown some signalling pathways that are targets of aspirin to inhibit tumor angiogenesis.** However, it warrants further study to verify the downstream of signalling transduction in these pathways.

It has been demonstrated that COX-2 in ECs is vital in regulating angiogenesis, and the microvessel density relate to the level of COX-2 expression (Jones et al., 1999; Kazuhiko et al., 2000). Moreover, in the sponge implanted mouse model, COX-2 mediated prostaglandins can up-regulate the expression of VEGF mRNA (Yoshida et al., 2003).

However, aspirin effectively inhibits COX-1 activity at a pharmacological concentration, but has a weaker active to block COX-2 activity (Meade et al., 1993; Mitchell et al., 1993). In fact, it has been demonstrated that salicylate inhibits Cyclooxygenase-2 transcription induced by IL-1 $\beta$  or phorbol 12-myristate 13-acetate by blocking C/Enhancer-binding Protein  $\beta$  binding to its cognate site on Cyclooxygenase-2 promoter (Michael et al., 2001). Furthermore, aspirin and sodium salicylate at pharmacological concentration blocked COX-2 protein expression induced by cancer and VEGF (Shtivelband et al., 2003) (Figure 2). COX-2 protein levels in HUVECs, inducing by PMA (Phorbolol 12-myristate 13-acetate), inhibited by Aspirin and sodium salicylate in the manner of concentration-dependent (Xu et al., 1999). Therefore, aspirin may inhibit COX-1 activity and COX-2 transcription to regulate angiogenesis.

### 2.3 Effect on platelet-mediated angiogenesis

Platelets are considered to be the main actors in each step of angiogenesis as they store various growth factors, proteases, cytokines, chemokines and cell adhesion molecules (Patzelt et al., 2012; Pipili-Synetos et al., 1998). Therefore, pharmacological interventions of platelet-associated proangiogenic activity have been recognized as an important adjuvant treatment for cancer (Bambace et al., 2011; Radziwon-Balicka et al., 2012).

The 15(S)-hydroxyeicosatetraenoic acid (15(S)-HETE), a direct COX-1 product in platelet, can combine with other pro-angiogenic factors released by platelets to regulate angiogenesis (Rauzi et al., 2016). Aspirin can block the angiogenic response of thrombin-stimulated human platelets in human microvascular endothelial cells (HMEC-1) (Etulain et al., 2013). In addition, aspirin inhibits 15(S)-HETE production in platelet with inhibition COX-1 to reduce angiogenesis, which also provide an explanation for the protective effects of low-dose aspirin on some cancers (Rauzi et al., 2016) (Figure 2). A study demonstrates that 15(S)-HETE can promote angiogenesis in HuDMECs through the PI3K-Akt-mTOR-S6K1 signaling pathway (Zhang et al., 2005). However, it warrants further study to verify the downstream of 15(S)-HETE signalling transduction when aspirin antiangiogenesis.

Platelet levels of thrombospondin-1 (TSP-1) have been indicated to regulate early stage of tumor angiogenesis (Zaslavsky et al., 2010). In breast cancer patients, taking aspirin therapy can upregulate TSP-1 level without a concurrent increase in VEGF levels in platelets (Holmes et al., 2013). It is also indicated that pretreatment with aspirin could reduce the amount of VEGF released from platelets at rest and after being

activated by ADP or interacting with MCF-7 cells (Battinelli et al., 2011). Furthermore, aspirin may correct abnormal platelet activation, prevent the release of a large number of angiogenesis regulators, and then help normalize the tumor vasculature and shape the tumor microenvironment, thereby reducing tumor invasion and progression (Su et al., 2014). There is a registered clinical trial to test the efficacy of aspirin use for exploring the platelet function of its mechanism of action, such as the change levels of COX-1, thrombospondin 1, NF-Kb and VEGF (Table1).

## 2.4 Effect on Hypoxia stimulation and GLUT1 expression

Neoplasms have been usually described as hypoxic structures, inefficient of oxygen transportation, and bearing twisted irregular vascular networks (Vaupel, 2004). Hypoxia-reoxygenation (HR), a major driver for tumor angiogenesis, stimulates proliferation of ECs via several signaling pathways including VEGF and transforming growth factor (TGF $\beta$ 1) (Khaidakov et al., 2011; Hu et al., 2008). Moreover, overexpression of TGF $\beta$ -R1 shows angiostatic effect, while blocking TGF $\beta$ -R1 can facilitate VEGF-mediated capillary formation and activation of genes related to angiogenesis (Liu et al., 2009). Aspirin effectively inhibits the angiogenic response involved in HR-mediated TGF $\beta$ 1 mRNA transcription and TGF $\beta$ 1-R1 upregulating, thereby inhibiting HR-stimulated tube formation in HUVECs (Khaidakov et al., 2011). In addition, aspirin can inhibit LOX-1-NADPH oxidase pathway in HUVECs to suppress HR-induced tube formation (Khaidakov et al., 2010).

Glucose transporter 1 (GLUT1) is the main route to uptake glucose in ECs (Goveia et al., 2014). The expression of GLUT1 is promoted in ECs by hypoxia, and the high expression of GLUT1 is related to neoplasm angiogenesis (Zapata-Morales et al., 2014; Semaan et al., 2011). In the process of switching from a quiescent state to angiogenic phenotype, glycolysis provides the essential energy and promotes sprouting of blood vessels during tumor-induced angiogenesis (Rivera et al., 2014). Interesting, aspirin can not only inhibit the expression level of GLUT1 mRNA and protein resulting in impaired glucose uptake ability, but also inhibit the intracellular lactate synthesis and ATP of SEND cells (rat vascular endothelial cell line) (Hu et al., 2014). Moreover, taking high-dose aspirin, even reaching 90 mM, does not produce side effects that interfere with glycolysis and glucose uptake in human erythrocytes (Worathumrong et al., 1975). Thus, aspirin inhibits the sprouting of blood vessels through regulating the expression level of GLUT1 and inhibiting lactate synthesis. However, it warrants further study to clarify the detailed mechanisms of aspirin in these phenomenon.

## 2.5 Effect on renin-angiotensin system

The renin-angiotensin system (RAS) is involved in a coordinated hormone cascade that regulates fluid-electrolyte balance and arterial pressure by controlling cardiovascular, adrenal and renal functions (Peach et al., 1997). Some studies suggest that angiotensin II (Ang II) in low concentrations can induce angiogenesis by activating angiotensin type 1 receptor (AT1R) and nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase (Skultetyova et al., 2007; Ushio-Fukai et al., 2006; Hu et al., 2007). MAP kinase activation is the downstream of NADPH oxidase, and inhibition of p38 and p44/42 MAP kinase can reduce angiogenesis (Hu et al., 2007; Seeger et al., 2010). Moreover, it has been noted that suppression of bradykinin degradation and angiotensin II(Ang II) synthesis in RAS can produce a net antiangiogenic state in tumorigenesis (Heffelfinger et al., 2007). Importantly, ASA and SA significantly reduce Ang II-induced expression of AT1R, VEGF, NADPH oxidase subtypes, p-p38 and p-p44/42 MAPKs in endothelial cells, and inhibit angiogenic effects of Ang II in capillary formation (Mitra et al., 2012). It warrants further study to research the effect of aspirin in renin-angiotensin system in clinical setting.

## 2.6 Effect on mononuclear phagocytic system

Within tumor microenvironment, cancer cells induce monocytes to differentiate for tumor-associated macrophages (TAMs) through recruiting monocytes by secreting chemotactic factors such as VEGF, macrophage chemoattractant protein-1 (MCP-1) and macrophage colony-stimulating factor (Solinas et al., 2009). Aspirin could disrupt the crosstalk of angiogenic cytokines between macrophages and 4T1 breast cancer cells (Hsieh et al., 2018). Macrophages, including categorization of M1 and M2 phenotype, have the

potentiality to convert the M1 into the M2 macrophages (Gordon et al., 2010; Spiller et al., 2015). M1 macrophages secrete factors that are involved in initiating the stages of angiogenesis, while M2 macrophages secrete factors are responsible for later process of angiogenesis (Spiller et al., 2014). Importantly, it has been shown that aspirin treatment raised the level of M1 marker expression, but decreased expression of M2 marker (Hsieh et al., 2018).

Thymidine phosphorylase (TP) is a proangiogenic factor that found to be chemotactic for ECs and to induce angiogenesis in vivo (Finnis et al., 1993; Miyadera et al., 1995). TP is highly expressed in macrophages of normal human tissues (Fox et al., 1995). Comparing with adjacent uninvolved tissues, TP is often overexpressed in solid tumors, and its expression is associated with higher microvessel count, increased tumor invasiveness and metastasis (Takebayashi et al., 1996). It also found that TP-expressing cancer cells secreted angiogenic factors (IL-8, bFGF and TNF $\alpha$ ) that stimulated endothelial cells migration and invasion (Elamin et al., 2015). Moreover, activated macrophages are one of the main sources for Tumor Necrosis Factor- $\alpha$  (TNF $\alpha$ ), and it has been suggested that the proangiogenic activity of macrophages is mainly mediated by TNF $\alpha$  (Beutler et al., 1986; Leibovich et al., 1987). Aspirin inhibits MCP-1 and interleukin-8 expression in TNF-alpha stimulated HUVECs (Yang et al., 2004). Besides, THP-1 monocytes induce TP expression by autocrine TNF $\alpha$ , and aspirin inhibits TP expression in THP-1 cells by reducing NF $\kappa$ B DNA-binding activity in the pathway of TNF $\alpha$ -induced TP expression (GENG et al., 2003). However, it warrants further studies to determine the downstream of TP signalling transduction in ECs when aspirin antiangiogenesis.

## 2.7 Effect on intercellular junctions and Pericytes

Disorganization of tumor vascular layers leads to increased permeability and leakiness, which is a common feature of tumor angiogenesis (Weis et al., 2011). The low vascularity, abnormal morphology, and high permeability of the tumor vessels leads to inefficient delivery of drugs and oxygen into tumors (Jain et al., 1998). Antiangiogenic drugs would be expected to inhibit the angiogenesis and may stop the growth of tumor tissues but not necessarily influence existing blood vessels or cause tumor shrinkage (Baluk et al., 2005).

VEGF, as a pro-angiogenic factor, not only plays a key role in inducing endothelial cell migration, proliferation and differentiation, but also can destroy tight junction proteins, thereby increasing vascular permeability and triggering tumor metastasis (Cross et al. 2013; Azzi et al., 2013). ASA significantly reduced the expression of VEGF in HUVEC, hTNF $\alpha$  reduced the VEGF release from endothelial cells of human primary glioblastoma and blocked the VEGF-promoted angiogenesis (Maity et al., 2019; Navone et al., 2018). Besides, salicylate decreased serum VEGF levels and reduced microscopic vascular structures in animal models (Ghezzi et al., 2004).

Moreover, three types of junctions in the endothelial monolayer have been described: adherens, tight and gap junctions (Bazzoni et al., 2004). The destruction of the adhesion junctions is a vital step in inducing angiogenesis and a prerequisite for releasing EC from the contact inhibition exerted by the association of VE-cadherin and  $\beta$ -catenin (Khaidakov et al., 2010). Immunocytochemistry performing to p120 catenin and VE-cadherin showed that adherens junctions in VEGF-induced HUVECs tube formation are severe disrupted, but the disruption effects is largely prevented by aspirin and salicylic acid with preincubated in cultures (Khaidakov et al., 2012). Furthermore, aspirin rescues vascular permeability and up-regulates the expression of VE-cadherin and Zonula Occludens-1 (ZO-1) in MDA-MB-231 conditioned medium (Maity et al., 2019). Aspirin also prevents disintegration of endothelial adherens junctions caused by Hypoxia-reoxygenation (HR) (Khaidakov et al., 2010).

Pericytes play a vital role in stabilization of blood vessel architecture and regulate vessel permeability (Bergers and Song 2005; Ribeiro et al., 2015). It has been suggested that aspirin can recruit pericytes from multipotent stem cells and help in binding with ECs, which is a sign of normalization of blood vessels and reduces permeability and leakiness through endothelial cell layer (Maity et al., 2018). Thus, normalized tumor vasculature may reinforce drug delivery to further solid tumors.

## 3. Antiangiogenic effects of ASA combination with actual proven therapies

The abnormal tumor vessels leads to inefficient delivery of drugs and oxygen into tumors (Jain et al., 1998). Thus, the normalization of the tumor vasculature by antiangiogenic drugs may reinforce other drugs delivery to further solid tumors, thereby producing an enhancement effect. As targeting angiogenesis is rising a great promise in patients as an antitumor therapy in combination with existing chemotherapies (Ma et al., 2008), we analyze antiangiogenesis properties of coadministration with aspirin.

The previous study has revealed that following coadministration with aspirin therapy, VEGF levels decreased in women with breast cancer relative to levels of receiving Tamoxifen therapy alone (Holmes et al., 2013). Combination therapy of ASA drugs with a VEGF inhibitor block angiogenesis more effectively than either agent alone (Huang et al., 2016). In addition, the administration of aspirin as an adjuvant in combination with Temozolomide, Bevacizumab and Sunitinib showed a synergistic effect on reduce capillary-like tube length in comparison to each exposure alone, in fact aspirin enhanced Temozolomide, Sunitinib and Bevacizumab effect, decreasing significantly Glioblastoma-endothelial cells VEGF-stimulated angiogenesis, especially at the high concentration (Navone et al., 2018). Also, the expression of VEGFR-2, HIF-1 $\alpha$ , anti-apoptotic BCL-2 and PI3K/AKT suffered a reduction after combined therapy of Temozolomide, Bevacizumab, Sunitinib and aspirin compared to single-drug treatment (Navone et al., 2018).

5-Fluorouracil (5-FU), a rationally synthesized antitumor agent, is widely used to treat several common malignant tumors, including cancer of the skin, colon and breast (Diasio et al., 1989). The expression of VEGF-A and VEGF-C is significantly reduced in the tumors treated with 5-FU combination with aspirin, and the antitumor effect of 5-FU is synergistically enhanced by aspirin (Zhang et al., 2013). There are three registered clinical trials involved in evaluate the antiangiogenesis efficacy of coadministration aspirin in treating cancers (Table1).

**Table 1** Clinical trials of aspirin in relation to antiangiogenic principles (data from ClinicalTrials.gov) \*

Study title	Condition (identifier)	Primary outcome measures	Secondary outcome measures	Phase	Study type or design	n
Acetylsalicylic Acid and Colorectal Cancer Prevention: Exploring the Platelet Function of Its Mechanism of Action	Colorectal Cancer (NCT02125409)	Assessment of the degree of COX-1 acetylation by ASA administered for 1 week.	1.Changes from baseline in different biomarkers; 2.Changes from baseline in eicosanoid generation in vivo by measuring urinary metabolites derived from COXs; 3.Changes in baseline platelet COX-1; 4.Change from baseline in plasma proteins of markers of angiogenesis; 5.Assessment of ASA plasma levels; 6.Changes from baseline of proteomic profile of selected angiogenesis factors, ie VEGF, FGF2, TGFbeta, EGF, PDGF, MMP, angiogenin, and angiogenesis inhibitors, ie endostatin, PF4, thrombospondin 1, alpha-macroglobulin, PAI 1 and angiotatin; 7. Change from baseline in eicosanoid biosynthesis and protein expression of markers of growth and progression of colorectal cancer (such as COX-2, NF-Kb and PI3K/Akt/mTOR pathway).	III	LPAR,OL	40
Assessment of Direct Biomarkers of Aspirin Action to Develop a Precision Chemoprevention Therapy of Colorectal Cancer	Colorectal Cancer (NCT03957902)	Assessment of changes in acetylation levels of COX enzymes in platelets and non-neoplastic and neoplastic colonic tissues	1.Assessment of changes in prostaglandin E2 (PGE2) levels in colorectal mucosa depending on drug dosis; 2.Assessment of changes in phosphorylated S6 protein (p-S6) levels in colorectal mucosa depending on drug dosis; 3.Assessment of changes in thromboxane B2 (TxB2) levels in urine as indirect systemic biomarker depending on drug dosis; 4.Assessment of changes in urinary metabolite 11-dehydro-TxB2 (TX-M) levels as indirect systemic biomarker depending on drug dosis; 5.Assessment of changes in major urinary metabolite of PGE2 (PEG-M) levels depending on drug dosis.	II	LSAR,OL	60
A Randomized, 2x2 Factorial Design Biomarker Prevention Trial of Low-Dose Aspirin and Metformin in Stage I-III Colorectal Cancer Patients	Tertiary Prevention in Colon Cancer (NCT03047837)	NFkB	1.pS6K, p53, beta-catenin, PI3K; 2.II-6, CRP, VEGF and HOMA index; 3.Gene expression levels; 4.Metformin concentration.	II	LFAR,Q	160
The Effect of Aspirin on Angiogenesis Proteins in Women on Tamoxifen Therapy	Breast Cancer (NCT00727948)	Changes in pro-angiogenic and anti-angiogenic protein levels	---	I	LSG,OL	35
Anti-Platelet and Statin Therapy to Prevent Cancer-Associated Thrombosis	Solid Tumor (NCT02285738)	Change in average sP-selectin levels	1.Frequency of major bleeding complications or clinically significant non-bleeding complications per patient; 2.Change in average Platelet Factor 4; 3.Change in average CD40 ligand; 4.Change in average serum thromboxane B2; 5.Change in average serum VEGF; 6.Change in average serum angiotensin-2; 7.Change in average serum hepatocyte growth factor; 8.Change in average serum PECAM; 9.Change in average serum PDGF; 10.Change in average plasma F1.2; 11.Change in average plasma TAT complexes; 12.Change in average plasma D-dimer; 13.Change in the number of thrombotic events.	I	LPAR,OL	17
Low-Dose Acetylsalicylic Acid in Treating Patients With Stage I-III Non-Small Cell Lung Cancer	Adenocarcinoma of the Lung (NCT01707823)	Change in PGE2 biosynthesis from baseline and at 14 days after discontinuation of a 7-day course of 325 mg ASA per day	Change in PGE-M levels from baseline and daily for 7 days after discontinuation of a 7-day course of 325 mg ASA per day	I	LSG,OL	4
Aspirin Mouthwash in Treating Patients With Oral Leukoplakia	Precancerous Condition (NCT01238185)	PGE2 levels as assessed by enzyme immunoassay; 2.COX-1 and COX-2 mRNA as assessed by qRT-PCR; 3.COX-1 and COX-2 protein expression as assessed by immunohistochemistry and quantified by immunoblotting to determine whether COX inhibition in oral dysplasia tissues is actually possible by use of the mouthwash formulation.	1.Comparison of WHO histological grade and clinical size of dysplastic lesions before and after treatment and assessment of proliferating cell nuclear antigen; 2.Tolerability and possible oral and other adverse effects using a questionnaire; 3.VEGF mRNA expression as assessed by qRT-PCR; 4.Protein expression as assessed by ELISA and immunohistochemistry for VEGFA; 5.Global gene response of dysplastic tissue as assessed by Affymetrix gene expression microarrays.	I	OL	40

L, interventional; NR, non-randomized; DB, double-blind SG, single-group; OL, open-label; CS, crossover; Ph, pharmacodynamics end point; R, Randomized; PA, Parallel Assignment; SA, Sequential Assignment; FA, Factorial; Q, Quadruple (Participant, Care Provider, Investigator, Outcomes Assessor)

#### 4. Antiangiogenic effects on novel derivatives of aspirin and ATL-1

Some less serious adverse events of aspirin are involved in stomach pain, vomiting, nausea and heart-burn

(Hua et al., 2019). Long-term aspirin therapy may induce gastrointestinal injury. Even low-dose aspirin can damage the gastroduodenal mucosa in varying degrees, including ulcers, erosions, and bleeding (Lavie et al., 2017). Another adverse event of aspirin is aggravating respiratory diseases such as asthma and chronic sinusitis with nasosinus polyposis (Pavón-Romero et al., 2017). Thus, in order to become greater safety and greater efficacy compared with ASA, the new derivatives of aspirin are needed.

The NO-donating non-steroidal anti-inflammatory drugs are expected to bring these two promising features for cancer prevention and treatment relative to traditional similar drugs (Yeh et al., 2004; Rigas et al., 2007). For NO-releasing aspirin, NO itself could induce COX2 expression (Basudhar et al., 2017), but aspirin may inhibit it thereby neutralizing the harmful effect of NO. In that way, it is expected that novel derivative of NO-releasing aspirin might show less gastrointestinal injury than aspirin. It has been demonstrated that NO-donating aspirin suppresses tumor angiogenesis by inhibiting VEGF expression and reducing the microvessel density in cancer mice xenografts (Ouyang et al., 2008).

PGE<sub>2</sub> as a downstream mediator play a major role in regulating angiogenesis, and suppression of its formation is involve in antiangiogenic effects (Méric et al., 2006). Co-ASS, Co<sub>2</sub>(CO)<sub>6</sub> alkyne derivatives of aspirin (Co-aspirin), significantly inhibited cellular PGE<sub>2</sub> formation from arachidonic acid in breast tumor cells (Ingo et al., 2009). Besides, the zebrafish embryos, an established model organism for studying angiogenesis and vascular development, treat with Co-ASS showed severe defects in angiogenesis, which is manifested in the lack or impaired formation of dorsal longitudinal anastomotic vessels and intersegmental vessels, as well as the reduction of intestinal veins (Lawson et al., 2002; Ott et al., 2009).

Moreover, aspirin-PC is another derivative of aspirin in which PC-enrichedsoylecithin is formulated with aspirin. In short-term clinical trials, the development of PC-related aspirin has been proved to be less harmful to the gastroduodenal mucosa because of its ability to protect the hydrophobic barrier property of the tissue surface (Cryer et al., 2011). Previous study demonstrate that treatment with aspirin-PC significantly reduce the level of VEGF and the number of tumor microvessels in ovarian tumor tissue, especially when aspirin-PC is used in combination with bevacizumab (Huang et al., 2016).

It has been shown that, when COX-2 acetylated by aspirin, the ability of generating prostanoids is blocked, yet acetylated COX-2 remains active in epithelial cells, mononuclear cells and endothelial cells and initiates the biosynthesis of new product of transcellular biosynthesis or cell-cell interactions termed as aspirin-triggered-15-epi-lipoxins (ATLs) (Clària et al., 1995). The previously studies reveal that 15-epi-16-(para-fluoro)-phenoxy-lipoxin A4 (ATL-1), an ATLs stable analog, inhibited VEGF-stimulated endothelial cell proliferation and migration in a concentration-dependent manner (Fierro et al., 2002). Moreover, VEGF-driven HUVEC migration requires the synergistic activation of two complementary pathways: one pathway involves the activation of SAPK2/p38 MAP kinase-mediated actin, and the other involves the phosphorylation of focal adhesion kinase (FAK) and the assembly of focal adhesions (Rousseau et al., 2000). Treatment of HUVEC with ATL-1 significantly reduced VEGF-induced p38 and FAK phosphorylation (Cezar-de-Mello et al., 2006). Besides, the multiple steps in the angiogenesis process also inhibited by ATL-1, including VEGF-induced EC proliferation, MMP-9 activity, HUVEC adhesion and VEGFR-2 phosphorylation (Cezar-de-Mello et al., 2008).

## 5. Conclusion

Based on these knowledge, aspirin is an intriguing and promising antiangiogenesis agent. We have addressed here the some potential antiangiogenic effects of aspirin therapy in not only decreasing endothelial cell proliferation and migration, but also in reducing macrophage or platelet-mediated angiogenesis. We have also discussed some mechanisms through which aspirin treatment may normalise existing blood vessels, including preventing disintegration of endothelial adheren junctions and recruiting pericytes, thereby reducing permeability and leakiness through endothelial cell layer. When a single-molecule is blocked, the tumor may bypass this angiogenic protein through overexpression of other angiogenic factors, resulting in a low therapeutic effect. The above drawback may be overcome by aspirin inhibiting several angiogenic molecules simultaneously, such as VEGF, cyclooxygenase, matrix metalloproteinase, and heparanase. Furthermore,

more clinical trials are needed to validate the antiangiogenic mechanisms and benefits of aspirin therapy, especially in combination with existing chemotherapies to prevent various human tumors, such as glioblastoma, breast cancer and colorectal cancer. However, angiogenesis is essential for reproductive function and wound healing, so the dose of aspirin should be quantified to inhibit tumor angiogenesis, but it does not injure healthy endothelial function and maintenance. The novel derivatives could be designed from aspirin backbone to be more antiangiogenic and more suitable as an antiangiogenesis agent while overcoming the side effects of aspirin.

### Conflict of interest statement

The authors declare no conflict of interest.

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