Controversy and Consensus

Cancer Research

Are Platelets the Primary Target of Aspirin's Remarkable Anticancer Activity?

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Introductory Statement

Aspirin, when administered at low doses, has emerged as a powerful anticancer drug due to both chemopreventive activity against many forms of cancer and its ability to block metastases when administered postdiagnosis. Platelets, which are often elevated in circulation during the latter stages of cancer, are known to promote epithelial–mesenchymal transition, cancer cell growth, survival in circulation, and angiogenesis at sites of metastases. Low-dose aspirin has been demonstrated to block this procarcinogenic action of platelets. In this article, we present evidence that aspirin's unique ability to irreversibly inhibit platelet cyclooxygenase-1 is a key mechanism by which aspirin exerts anticancer activity.

Introduction

One of the oldest and least appreciated observations in the field of oncology, is that of Armand Trousseau in 1865 that patients with late-stage cancer frequently develop thrombosis/thrombophlebitis—which is now a syndrome bearing his name (1). This pioneering observation predated the discovery of platelets in 1882, which are anucleated blood cells originating from megakaryocytes. Platelets are best known to promote hemostasis and thrombosis and have gained attention in oncology due to their role in cancer progression (2). Patients at the latter stage of cancer affecting the breast, colon, gastric, lung, ovary, and prostate often have thrombocytosis (elevated platelet counts; ref. 2). Furthermore, the efficacy of chemotherapy is attenuated in patients with cancer with high platelet counts. Tumor-educated platelets display altered RNA profiles and their potential value as biomarkers using analytics called "liquid biopsies" is currently being explored (3). Thus, the fundamental question is the importance of platelets to the progression and metastatic spread of cancer versus their elevation in circulation being a secondary consequence of the disease itself.

Aspirin, the first nonsteroidal anti-inflammatory drug (NSAID) discovered (in 1897) and widely used to reduce fever, pain, and platelet activity, has emerged as a powerful agent in secondary prevention of cancer including colorectal cancer (4, 5). Aspirin has the unique capability of inhibiting both isoforms of cyclooxygenase (COX-1 and COX-2) to reduce the synthesis of eicosanoids, which will be subsequently converted via cell-specific synthases into biologically active prostaglandins (PGE_2, $PGF_{2\alpha}$, PGI_2) and thromboxane A_2 (TXA_2). Of particular importance, to both its cardiovascular and cancer indications, is the drug's unique ability to irreversibly inhibit platelet COX-1 (via acylation of serine 530), preventing platelet activation over the remaining lifetime of the affected platelets. Aspirin also shares the ability with other NSAIDs to nonspecifically inhibit COX-2, which is overexpressed in many cancers as reported by Tsujii and colleagues (6) Because PGE_2 promotes tumor proliferation, differentiation, and angiogenesis, it is widely considered that the anticancer effect of aspirin and related NSAIDs resides primarily in their COX-2–inhibitory activity, which is supported by preclinical and clinical evidence that COX-2–selective inhibitors (coxib) possess chemopreventive activity.

In this article, we will present our perspective that the primary target of low-dose aspirin's anticancer activity is platelet COX-1, thereby preventing platelets from inducing epithelial–mesenchymal transition (EMT) and the downstream procarcinogenic responses including the induction of COX-2. Thus, we feel this action of low-dose aspirin should be studied more broadly to further elucidate its contribution and that of related antiplatelet agents, in cancer prevention/therapy. Our perspective of highlighting the antiplatelet action of aspirin should not be interpreted as a dismissal of the evidence supporting the contribution of COX-2 to cancer progression and the potential utility of coxibs in cancer prevention/therapy, as an appreciation of both COX isoforms is important.

Evidence that platelets promote cancer cell proliferation, migration, survival, and metastasis

A compelling body of evidence can be found in the scientific literature, which supports the role of platelet–cancer cell interaction in the progression of cancer from its induction at sites of inflammation to the development and growth of metastases, which will be reviewed below. A bidirectional signaling crosstalk occurs between platelets and tumor cells in both the peripheral circulation and in the tumor microenvironment (2, 7). Tumor cells can induce platelet aggregation, promote secretion of platelet granular contents, and generate platelet microparticles. Tumor-derived IL6 stimulates megakaryopoiesis, which supports

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thrombocytosis. Conversely, platelets promote cancer cell growth, EMT, and help evade immune attack (2, 7). Intravasation of circulating platelets into tissues within the tumor microenvironment undergoing inflammation and dysplasia can redirect cell properties to support procarcinogenic changes. Both cell culture and animal experiments suggest, eicosanoids like PGE_2 (generated by tumor COX-2) and TXA_2 (generated by platelet COX-1; ref. 8) promote cancer cell growth and proliferation. Platelets facilitate angiogenesis by secreting proangiogenic factors like VEGF, platelet-derived growth factor, basic fibroblast growth factor from their α granules. Platelet-derived TGFβ/SMAD signaling induces EMT in a diverse group of tissues and in doing so, increase migration and invasive activity required for tumor metastasis. Platelet-derived chemokine CCL-5/RANTES upregulates IL8 in breast cancer cells to promote tumor invasion (9). VEGF also promotes immunosuppression of cancer cells by facilitating the recruitment of tumor-associated macrophages, myeloid-derived suppressor cells, and T-regulatory cells to the tumor microenvironment. Upon gaining access into circulation, tumor cells rely on platelets to overcome detachment-induced apoptosis (anoikis) by activating Yes Associated Protein 1 signaling in tumor cells (2). Furthermore, platelets protect tumor cells from immune attack by suppressing T cells via the platelet TGFβ/GARP (glycoprotein A repetitions predominant) axis (10). Platelets contribute to neutrophil extracellular traps that can engulf surrounding circulating cancer cells and thereby prevent attack by NK cells and macrophages in blood. Finally, platelet receptors facilitate cancer cell attachment to distal endothelium and mediate their arrest in the vasculature for metastasis. Thus, blood platelets can induce major changes in target tissues by promoting a transformation that can lead to dysplasia, cancer growth, and metastatic spread. These preclinical observations suggest fertile opportunities for antiplatelet agents in cancer prevention and treatment.

Aspirin as a powerful anticancer agent

It is well established that aspirin inhibits platelet reactivity by blocking COX-1 activity irreversibly and reducing TXA_2 production and because of this relationship low-dose aspirin drug is widely employed in the management of cardiovascular disease. Consistent with the potential role of platelets in various stages of cancer progression in murine models, inhibition of platelet reactivity by aspirin attenuated: platelet-mediated proliferation of tumor cells (8), adhesion of tumor cells to the endothelium (11), aggregation of platelets on tumor cells (11), invasion of tumor cells facilitated by tumor IL8 (9), and translocation and EMT of tumor cells (8, 12). Aspirin also inhibits angiogenesis, which promotes cancer growth and metastases by markedly reducing the production/secretion of platelet proangiogenic factors like 15(S)-hydroxyeicosatetraenoic acid (HETE) and VEGF. Lastly, aspirin has been reported to enhance cancer immunotherapy by blocking the immunosuppressive action of platelets on T-cell function (10).

A large number of observational studies in humans have strongly indicated that regular aspirin consumption is associated with a significant 20%–40% reduction in the incidence of as many as 20 different types of solid cancers with the strongest evidence for a survival advantage of chronic low-dose aspirin users against deaths attributable to colorectal cancer, ovarian, and breast cancer (4, 5, 13). These conclusions are primarily based on long-term data from the Nurses Health Studies (NHS) I-III. This compelling association between aspirin use and colorectal cancer led the US Preventive Services Task Force to officially recommend in 2016 that older subjects (>50 years for males and >60 years of age for females) who have the greatest risk of cardiovascular disease should take low-dose aspirin on a regular basis to reduce the risk of both myocardial infarcts and colorectal cancer(14), under the proviso that they consult their physicians due to the fact that aspirin will cause GI ulceration and bleeding in sensitive individuals. It is also important to note that not all the studies have obtained positive data on aspirin's chemopreventive activity, notably recent findings of the Aspirin in Reducing Events in the Elderly (ASPREE) trial that subjects >70 years of age who took low-dose (100 mg) enteric-coated aspirin on a daily basis showed no clinical benefit and in fact had a modestly higher death rate overall, which unexpectedly was attributed mostly to cancer-related mortality, that even surpassed deaths due to major hemorrhage (15) The reasons for these conflicting findings in the literature are uncertain, but may, in part, be explained by differences among the studies, with regard to whether the study was designed as a Primary (ASPREE) or Secondary Prevention trial; the age of the subjects at the initiation of the study (ranging from 30 years in NHS to 70 years in ASPREE); the regularity of taking aspirin (once or twice/weekly vs. 5–7 times/week); the aspirin dose consumed, which ranged from 75 mg to 600 mg/daily, and the dose form (enteric-coated vs. immediate-release aspirin).

The life time of platelets is approximately 7 days and this may be further shortened during conditions of inflammation. In this context, if the major target of aspirin is platelets, then we argue that the consumption of NSAID only once/twice a week may lead to an increase in the subpopulation of platelets in circulation that are not inhibited by aspirin. In a recently published study, Lucotti and colleagues (11) provided compelling evidence using transgenic mouse models that, similar to the COX-1–selective inhibitor SC-560, aspirin reduced lung metastases in mice primarily by inhibiting platelet COX-1, the generation of thromboxane, and the activation of platelet/cancer cell cross-talk. In contrast, inhibition of COX-2 did not prevent metastatic colonization. Furthermore, aspirin has a short half-life in circulation of <15 minutes, as it is rapidly metabolized to salicylic acid. Unlike aspirin, salicylic acid is a weak inhibitor of COX (requires millimolar range). On the basis of the pharmacokinetic studies, lower doses (75–100 mg) of orally ingested aspirin should be sufficiently effective to selectively inhibit platelet activation, as platelets cannot resynthesize COX-1. On the other hand, cancer cells can resynthesize COX-2, and therefore it is likely that the dose of circulating drug may not mount sustained inhibition of COX-2 (16). It, therefore, will be of interest to test whether chronic consumption of low-dose aspirin results in sustained concentrations of acetylsalicylic acid in circulation, which are sufficient to induce long-term repression of COX-2 in cancer cells as well. However, it should be noted that high doses of the drug in addition to inhibiting COX-2 may also have unanticipated targets due to nonspecific protein acylation, which is a characteristic of aspirin.

The central and most provocative question for oncologists is whether aspirin administration postcancer diagnosis has significant efficacy as a Secondary Preventive anticancer agent in reducing the growth, recurrence, and metastatic spread of cancer. Building on the above and other preclinical studies that aspirin treatment was effective in reducing tumor size and metastases, a number of outcome studies have focused on postdiagnostic use of aspirin, in most cases as an adjunctive therapy. Most studies have

demonstrated encouraging results, that the recurrence and/or metastatic spread of a number of cancers (colorectal, gastroesophageal, ovarian, breast, and prostate cancer) were reduced in subjects who took aspirin postdiagnosis alone or together with other chemotherapeutic agents (17). In addition, consumption of aspirin has been reported to be significantly associated with a decrease in cancer-associated mortality and a prolongation of life (4, 17, 18). Based upon these observational findings, several prospective clinical trials are now recruiting patients that have an arm, where patients take aspirin either alone or as an adjunctive therapy after they were diagnosed with: breast cancer (Alliance trial); four solid cancers [colorectal cancer, breast, gastroesophageal, or prostate cancer (ADD-ASPIRIN; ref. 19]; or subjects with Lynch Syndrome who have a genetic risk of developing colonic polyps or colorectal cancer (CAPP2). Although these studies are directing patients with cancer to take aspirin at different doses ranging from 100 mg (ADD-ASPIRIN) to 300 mg (ADD-ASPIRIN and Alliance) to 600 mg (CAPP2), it is our recommendation that future studies focus on low-dose immediate-release aspirin, as enteric coating appears to reduce the bioavailability of the drug. In support of using aspirin at low dose, Rothwell and colleagues who first reported the efficacy of aspirin to reduce cancer metastases and mortality postdiagnosis (17) have recently reported that low-dose aspirin (75 mg) was most effective at reducing colorectal cancer–related mortality in nonobese patients (18). Thus, the aspirin dose most selective to irreversibly inhibit platelet activity appears to be the most effective anticancer dose. Lastly, in support of platelets being the primary target of the drug's anticancer action, preclinical and clinical studies have reported that platelet ADP P2Y12 receptor antagonist, clopidogrel/Plavix possesses chemopreventive properties (20). Furthermore, antiplatelet agents such as DG-041[PGE2 EP3 receptor blocker] and ticagrelor (a P2Y12 receptor antagonist) have been reported to block EMT (12). However, use of aspirin in cancer management is not without challenge. Variable resistance to aspirin in patients with cancer could potentially confound the outcome. The effective dose of the drug should be able to balance the ability to block cancer growth with minimal changes in hemostasis.

Conclusion

In our opinion, there is emerging evidence to support platelets as a key factor in the progression of many cancers, and the major target of low-dose aspirin is platelet COX-1 as opposed to cancer cell COX-2. On the basis of these considerations, oncologists should be closely monitoring patients with stage II or III cancers for a spike in circulating platelet number over the course of their illness, as potential biomarkers of metastases. We also feel a compelling case can be made for investigators to monitor COX-1 expression in tumor tissue/cells as diligently as they have for monitoring the expression of COX-2, as several studies have indicated that both COX isoforms are expressed in a number of cancers, although the majority of studies have focused only on COX-2 (16). Lastly, we recommend that consideration should be given by oncologists to include aspirin in the management of patients diagnosed with various forms of cancer for secondary prevention of cancer recurrence and metastases.

Disclosure of Potential Conflicts of Interest

L.M. Lichtenberger has ownership interest (including stocks and patents) in PLx Pharma Inc. No potential conflicts of interest were disclosed by the other authors.

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