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Anti-platelet treatments in cancer: Basic and clinical research

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ABSTRACT

Over the past few decades the central role that platelets play in cancer development and progression, and especially in metastasis, has been elucidated. The molecular mechanisms responsible for initiating and mediating tumor cell-induced platelet aggregation and secretion have been largely unravelled. Considerable mechanistic insight into how platelets contribute to tumor angiogenesis, immunoevasion and cancer cell invasion have been clarified and, consequently, platelets have been identified as potential new drug targets for cancer therapy.

This article gives an overview of the platelet-targeted pharmacologic approaches that have been attempted in the prevention of cancer development, progression and metastasis, including the application of antiplatelet drugs currently used for cardiovascular disease and of new and novel strategies.

1. Introduction

The involvement of platelets in cancer growth and metastasis is a longstanding concept. An inverse correlation between platelet count and disease-specific survival has been described for several cancers and numerous basic and clinical research observations show that platelets affect disease burden and treatment efficacy in cancer patients, and participate in cancer metastasis.

Platelets physically and functionally interact with various tumor cells through surface receptors including integrins. β1 integrins and β3 integrins in particular participate in platelet–tumor cell interaction and in tumor metastasis [1].

Platelets display their pro-metastastic role through the secretion of pro-metastatic factors (e.g. autotaxin), chemoattraction of granulocytes to platelet/tumor aggregates, activation of epithelial-mesenchimal-like transition (EMT) via direct contact with tumor cells and/or secretion of TGFβ1, release of microvesicles containing microRNA-223 which targets the tumor suppressor EPB41L3, adhesion to tumor cells providing physical protection from direct contact with NK cells [2].

The interplay between platelets and tumor cells involves the formation of platelet–tumor cell aggregates in the circulation, with platelets forming a shield for tumor cells allowing the latter to escape natural killer (NK) cell and tumor necrosis factor (TNF)α cytotoxic activity. Platelets may also help tumor cell extravasation to the metastatic niche. On the other hand platelet activation releases platelet-derived growth and proangiogenic factors that may, contribute to tumor growth and angiogenesis.

Platelets flowing through the tumor vasculature get activated, adhere to the neovascular endothelium, aggregate and become part of the tumor's microenvironment, thus potentially influencing the tumor parenchyma and stroma of most solid tumors, particularly carcinomas. Platelets contribute to tumor persistence in the circulation by shielding tumor cells from destruction by NK cell [3–5] and suppress IFN-γ secretion by NK through the interaction between glucocorticoid-induced TNF-related ligand (GITR), a member of the TNF receptor superfamily that acts as a NK-inhibitory ligand on the surface of NK and its ligand (GITRL) present on platelet's surface [10–14]. Platelets contribute to metastasis and distant tumor growth by aiding tumor cell attachment to the endothelium and by releasing angiogenic and growth factors, such as vascular endothelial growth factor (VEGF) [6,7] and transforming growth factor-β (TGF-β) [8,9].

Tumor cell invasion through the extracellular matrix (ECM) is a crucial step in tumor metastasis, and this process is mediated by proteolytic enzymes, such as matrix metalloproteinases (MMPs), that degrade the ECM surrounding blood vessels to allow cancer cells to penetrate in tissue. Indeed, the ECM surrounding blood vessels plays a critical role in the limitation of extravasation and intravasation of tumor cells. Platelet-derived TGF-β1 is crucial in inducing tumor growth and metastasis by up-regulating MMP-2 and MMP-9 [15] and by activating the TGFβ/SMAD and NF-kB pathways in cancer cells [16].

The importance of platelets in cancer growth is supported by the finding that platelet depletion results in a marked reduction of tumor growth in an orthotopic model of ovarian cancer, in vivo models of experimental pulmonary metastasis and in a murine model of spontaneous metastasis [17].

The interaction between cancer cells and/or the neovessels

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irrorating tumors and platelets leads to the triggering of adhesion and activation of the latter, a process also called tumor-cell induced platelet aggregation (TCIPA). TCIPA is mediated by multiple agents: tissue factor (TF), thrombin, adenosine diphosphate (ADP), TxA2, and MMP [18].

Therefore the use of antiplatelet agents as adjuvant cancer therapy or the exploitation of platelets as carriers of antitumor agents are attractive approaches.

2. Cyclooxygenase inhibitors

Cyclooxygenase (COX) is the enzyme transforming arachidonic acid into the precursor (PGG_2-PGH_2) of different prostaglandins (PGs) or of thromboxanes (Tx), depending on the cell type.

Arachidonic acid is cleaved from membrane phospholipids by phospholipase A_2 and is then transformed by cyclooxygenases (COXs) into PGH2, which is rapidly converted into prostanoids by different PG- or Tx-synthases [19]. The COX-1 isoenzyme preferentially couples with TXA_2 synthase (in platelets) and PGF2 synthase (PGFS), whereas COX-2 is associated mainly with PGI_2 synthase (PGI-S) (in endothelial cells) and PGE synthase (PGEs) in several cell types, including mucosal cells of the gastrointestinal tract [20]. Evidence exists for an antitumor activity of aspirin, which acts as an active-site acetylating agent inducing an irreversible inactivation of COX-1 (through acetylation of Ser 529) and COX-2 (through acetylation of Ser 516), with the consequent suppression of prostaglandin and thromboxane production [21,22].

The inhibition of platelet TxA_2 production by aspirin is the basis of the most widely diffused antiplatelet therapy in clinical use.

2.1. Preclinical studies

Early observations showed that tumor metastases were reduced in rats treated with aspirin [23] and that prostaglandin concentration was raised in rat colorectal tumor tissue [24,25], opening the way to the studies on a potential benefit of aspirin in cancer. Supporting observations showed that oral administration of aspirin significantly inhibited the incidence and number of invasive, azoxymethane-induced adenocarcinomas of the colon in rats [26] as well as the onset of lung tumors induced by a tobacco-specific nitrosamine in mice [27]. Aspirin, administered to mice at a dose equivalent to low-dose in humans (around 100 mg/day), induced apoptosis of colorectal cancer (CRC) cells by a mechanism involving the downregulation of the IL-6-STAT3 signalling pathway [28]. The antitumor activity of aspirin is considered to be driven by the inhibition of COX-2, which is overexpressed in cancer cells [29,30], but also by the inhibition of platelet adhesion to tumor cells, and of TCIPA, essential steps in the protective effects by platelets from immune surveillance against cancer cells [31]. CRC cells (HT29) previously exposed to platelets in vitro transform into mesenchymal-like cancer cells which, injected into the tail vein of immunodeficient NOD-SCID mice, produce a higher number of lung metastases compared to HT29 cells not exposed to platelets, an effect associated with enhanced systemic biosynthesis of TXA_2 and PGE_2 in vivo. Aspirin administration prevented the increased rate of metastasis as well as the enhanced production of TXA_2 and PGE_2 induced by platelet-primed HT29 cells [32].

2.2. Clinical studies

From a case-control study, chronic aspirin use among CRC-patients was significantly lower than among age/sex-matched non-CRC-controls [33]. Later, a meta-analysis cumulating data from several case-control studies in CRC patients, involving > 30,000 subjects, concluded that regular aspirin use was associated with a reduced risk of CRC (pooled odds ratio (OR) 0.62, 95%CI 0.58–0.67, $p < 0.0001$) [34]. A few small randomized, double-blind, placebo-controlled trials, involving around 3000 participants, evaluating chronic aspirin for the secondary prevention of CRC [35–38], cumulatively showed a significantly reduced risk of developing an adenoma on follow-up (0.83, 95%CI = 0.72 to 0.96, $p = 0.012$) [39]. In carriers of hereditary predisposition to non-polyposis colon cancer aspirin was shown to prevent the development of cancer [40] and to prolong survival when started after diagnosis [41]. Given that in most of these trials high doses of aspirin were used, the doubt that the effect of aspirin would be essentially due to an action on tumor cell COX-2, requiring higher doses, and that therefore long term platelet-selective low-dose, aspirin would not prevent CRC, was risen. Therefore, a follow-up cumulative observation study of four large randomized trials on aspirin (75–300 mg/daily) versus control in primary and secondary prevention of vascular events, involving over 14,000 patients followed-up for a median of 18.3 years, was performed showing that allocation to aspirin significantly reduced the 20-year risk of colon cancer (HR 0.76, 95%CI 0.6- to 0.96, $p = 0.02$) and cancer-related mortality (HR 0.65, 95%CI 0.48 to 0.88, $p = 0.005$), but not that of rectal cancer, and that the benefit increased with treatment duration such that at least 5 years of treatment were required to reduce colon cancer risk. Moreover, benefit was evident with aspirin doses as low as 75 mg/day, with no further increase of benefit with greater doses [42]. This is an important observation because it is known that the risk of major bleeding with aspirin is dose-dependent [43,44].

Other subsequent meta-analyses of individual patient data from large aspirin studies of aspirin in cardiovascular prevention showed that aspirin reduced mortality also from non-gastrointestinal cancers [45], prevented metastasis from all cancers [46] and that the effect was only evident from 5 years of continued treatment onward [47]. These conclusions have been criticized because of the retrospective nature of the analysis on non-pre-specified end-points, because most of the recorded cancer events occurred well after the end of the randomized follow-up phase of the studies, and because some important trials, like the Women's Health Study, had been excluded from the analysis. Therefore, several ad hoc designed adjuvant trials of low-dose aspirin have been initiated in the last few years, in patients with various types of newly diagnosed cancer, and the protocols of some large, ongoing, primary cardiovascular prevention trials with aspirin (ASCEND, ACCEPT-D, ARRIVE and ASPREE) have been modified to collect prospective information about cancer incidence. The results of these ongoing studies will finally provide prospective evidence on the role of chronic aspirin in chemoprevention.

3. PDE inhibitors

Phosphodiesterases (PDEs) catalyze the hydrolysis of cyclic (c) AMP and cGMP, two powerful intracellular inhibitory second messengers, to inactive 5′AMP and 5′GMP. Of the over 60 different PDEs known, platelets express PDE2, PDE3, and PDE5 and their inhibition rises the intraplatelet levels of the two cyclic nucleotides thus inhibiting platelet activation [48].

Among the isoenzyme-selective PDE inhibitors developed as antiplatelet agents, cilostazol and dipyridamole have been explored as possible adjuvant therapy in cancer.

3.1. Cilostazol

Cilostazol (6-(4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy)-3,4-dihydro-2(1H)-quinolinone) is a specific and strong inhibitor of PDE3 in platelets and smooth muscle cells, it is in clinical use since 1999 for the treatment of intermittent claudication [49] and has shown protective effects on ischemic cardiovascular events [48]. Its effect on cancer has been only evaluated in preclinical studies.

3.1.1. Preclinical studies

Cilostazol suppressed the migration of human colon cancer cells, DLD-1, induced by soluble fibronectin or fetal bovine serum in a