



Research paper

The role of platelets and megakaryocytes in bone metastasis

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ABSTRACT

Blood platelets have been known for more than a century as important partners for successful metastatic dissemination of solid tumors. Cancer cell-induced platelet activation is a key event responsible for prometastatic activity of platelets. Blocking platelet aggregation inhibits the progression of skeletal metastases through mechanisms that are not fully understood. The establishment and progression of bone metastases are strongly influenced by the bone remodeling process. Growth factors and cytokines released upon platelet activation may contribute to both skeletal tumor growth and osteolytic lesions. Megakaryocytes are platelet precursors located in the bone marrow that control bone mass through direct stimulation of osteoblast functions and indirect inhibition of osteoclast activities. Considering growing evidence for their role in the metastatic cascade, platelets and/or megakaryocytes may provide new therapeutic opportunities to help limit bone metastases.

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1. Platelets and cancer metastasis

Blood platelets play a primary role in wound healing and hemostasis. Since the first observation made (on himself) by the surgeon A. Trousseau in the late 1800s, thrombosis is now accepted to be associated with poor cancer outcomes. Aspirin prevents distant metastasis of adenocarcinomas that accounts for the early reduction in cancer deaths in trials of daily aspirin *versus* control [1]. Clear demonstration of platelets controlling metastasis emerged in the mid-1900s through platelet depletion experiments that remarkably inhibited the incidence of lung metastases in mice [2]. Therefore, platelets are now considered as preeminent factors of cancer metastasis. Metastatic tumor cells induce platelet aggregation and embolus formation. In turn, platelets release a plethora of factors that contribute to circulating tumor cell (CTC) survival in a stressed condition generated in the bloodstream. Shear stress and Natural Killer cytotoxic cells are the main threats to CTCs inside the blood vessels [3]. At later steps of the metastatic cascade, interaction of CTCs with platelets favors extravasation and seeding of CTCs to distant organs. Platelets and endothelial cells express several types of selectins that support transient CTC adhesion to the vessel wall [4]. Plasma fibrinogen and its platelet-

specific receptor, $\alpha\text{IIb}\beta\text{3}$ integrin, also contribute to platelet-CTCs emboli [5]. Both members of the β3 integrin family, platelet $\alpha\text{IIb}\beta\text{3}$ and tumor $\alpha\text{V}\beta\text{3}$, are involved in CTC adhesion and invasion under flow conditions [6]. In addition to contribute to multiple steps of the metastatic cascade, platelets are also important modulators of inflammation and angiogenesis. These aspects of platelet functions are outside the scope of the present review but have been very well described in recent publications [7,8].

2. Platelets and bone remodeling

Clinical studies focused on the role of plasma rich platelets (PRP) demonstrated the involvement of thrombocytes in bone remodeling under acute and physiologic conditions [9]. Platelet aggregates localize around the sites of fractures or micro-fractures, wherein they release intragranular growth factors such as Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), Insuline-Like Growth Factor 1 (IGF-1) or Transforming Growth Factor 2 ($\text{TGF}\beta$), all known to recruit osteogenic cells [10]. Thromboxane A2 (TxA2) and Prostaglandins were also identified as bone remodeling modulators [9]. The role of platelets in osteoclastogenesis and bone resorption remains controversial. Several studies showed that activated platelets and PRP can enhance osteoclastogenesis via a RANKL-dependent mechanism [11], whereas others showed inhibitory effects [12]. Such discrepancies

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might derive from complex experimental settings using functional platelets *in vitro*.

3. Platelets and bone metastasis

It is now well established that platelets are essential for cancer metastatic dissemination and progression to the bone, as revealed by the seminal work using $\beta 3$ -deficient mice that exhibited a 95% decrease in skeletal tumor burden after intracardiac inoculation of B16 melanoma cells [13]. The use of osteoclast defective $src^{-/-}$ mice and specific platelet aggregation inhibitors could discriminate between the roles of $\beta 3$ integrins of osteoclasts and of platelets. Integrin $\alpha IIb\beta 3$ controls both platelet aggregation and CTC homing to the bone microenvironment, whereas osteoclast integrin $\alpha V\beta 3$ drives bone osteolysis [13]. Our group extended the role of platelet $\alpha IIb\beta 3$ integrin, not only to the onset of skeletal metastasis but also to the progression of bone metastasis, by treating metastasis harboring mice with integrilin, a pharmacological $\alpha IIb\beta 3$ antagonist [14]. This study also revealed the role of platelet-derived lysophosphatidic acid (LPA) as an enhancer of bone metastasis in metastatic breast cancer models. LPA is a bioactive lipid that exhibits growth factor-like activities by promoting proliferation, survival migration and the secretion of pro-osteoclastic cytokines [Interleukin 6 (IL-6) and 8, (IL-8), monocyte chemoattractant protein 1 (MCP-1), chemokine (C-X-C motif) ligand 1 (Gro α)] [14]. LPA is the final product of the lysophospholipase D activity of autotaxin (ATX) that controls the physiological levels of LPA in blood. We recently demonstrated that nontumoral circulating ATX can be stored in α -granules of resting platelets and released upon breast cancer cell stimulation contributing to metastasis formation [15].

Several anti-platelet therapies have been evaluated in the context of bone metastasis. *In vitro*, a dual anti-platelet treatment using the soluble ADPase APT102 and an inhibitor of TxA2 synthesis (Aspirin) markedly affected tumor cell-induced platelet aggregation without altering tumor cell viability. While *in vivo*, ATP102 and Aspirin treatment did not affect primary tumor growth, the combined treatment significantly attenuated the melanoma and breast cancer bone metastasis formation in mice [16].

4. Megakaryocytes and bone mass acquisition

Mature Megakaryocytes (MKs) are located in the bone marrow in vascular sinusoid areas where they undergo changes leading to the production of platelets [17]. Knockout mice for the transcription factors Nf-E2 and GATA-1 have marked and mild thrombocytopenia, respectively, which in both cases are associated with an increase in MK number revealing that Nf-E2 and GATA-1 are required for MK terminal differentiation and platelet production [18]. Intriguingly, both Nf-E2 $^{-/-}$ and GATA-1 $^{-/-}$ mice exhibit high bone mass phenotypes increased [Bone Mineral Density (BMD), Bone Volume over Tissue Volume ratio (BV/TV), trabecular and cortical thicknesses]. MKs produce a large variety of factors, among them are bone regulators such as [Bone Morphogenetic Protein-2,-4,-6 (BMP-2,-4,-6) and TGF β] [19], which may account for MK-stimulating bone formation. On the other hand, osteoblasts secrete multiple cytokines that control hematopoiesis [Leukemia Inhibitor Factor (LIF), Stem Cell Factor (SCF) and IL6] including MK proliferation and differentiation [20]. Consequently, MK and osteoblasts could stimulate each other, establishing a “win-win” situation at localised bone sites. Osteoblast proliferation is enhanced by MKs via a process regulated in part by integrin signaling ($\alpha 3\beta 1$, $\alpha 5\beta 1$, $\alpha IIb\beta 3$) controlling subcellular localization of the tyrosine kinase Pyk2 in osteoblasts [21]. MKs also produce the physiological osteoclast inhibitor Osteoprotegerin (OPG) that could explain the

inhibitory effect of MK conditioned media on osteoclastogenesis. However, conditioned media collected from MKs derived from OPG $^{-/-}$ mice also provides inhibition, thus minimizing the contribution of OPG in the endogenous *anti*-osteoclastic activity of MKs but confirming the secretion of unidentified osteoclast inhibitors by MKs [22].

5. Megakaryocytes and bone metastasis

Thrombopoietin (TPO) is a master regulator of Megakaryocytopoiesis and platelet production [17]. Transgenic mice overexpressing TPO exhibit an increase in MK number in the bone marrow and develop a high bone mass phenotype [23]. TPO inhibits osteoclast differentiation and their resorption activity *in vitro*. Thus, *in vivo* TPO might inhibit osteoclast function both directly and indirectly through increased production of MK-derived osteoclast inhibitors for inducing osteoclerosis [24]. The increased expansion of MKs in BALB/c nude mice in response to a 5-day TPO administration prior to the intracardiac injection of PC3 prostate cancer cells remarkably decreases the extent of skeletal lesions and tumor burden [25]. This unexpected result may be because TPO primarily increases the production of platelets by MKs and thrombocytosis create a more favorable metastatic environment for PC3 cells in the circulation. The platelet-production-independent function of MKs might predominate in this phenomenon. Mouse primary MKs inhibit proliferation of prostate carcinoma cell lines (PC3, C4-2b, VCaP) in *in vitro* co-culture systems. Intercellular contacts between MKs and PC3 cells lead to down-regulation of cyclinD1 in cancer cells and upregulation of pro-apoptotic factors (such as ASC and DAPK1), favoring apoptosis and inhibition of skeletal tumor growth. Therefore, the anti-tumor effect of MKs in synergy with their *anti*-osteoclastic action is likely the reason for inhibition of PC3 cell bone metastasis development. Nonetheless, such an anti-metastatic activity of MKs has not been reported in other types of organs and tissues. In contrast to their action on prostate cancer cell lines, MKs stimulate the proliferation of osteosarcoma cells [25]. Further experimental work is required to determine whether the anti-bone metastasis activity of MKs could be generalized to other types of solid tumors.

Intravenous injection of B16F10 melanoma cells in Nf-E2 $^{-/-}$ mice produces markedly less lung metastatic lesions compared to that in wildtype mice [5]. This provides one of the strongest confirmations for the role of platelets in hematological dissemination of tumor cells although bone tissues were not examined. The platelet-type von Willebrand disease (Pt-vWD) is characterized by a higher affinity of platelets to the soluble form of von Willebrand factor due to a point mutation in GPIIb α to a persistent thrombocytopenia and a bleeding disorder. Transgenic mice reproducing the Pt-vWD exhibit increasing number of MKs and a high bone mass phenotype [26]. Due to its specific expression in MKs and platelets, GPIIb α has been suggested as target for the development of new therapies against skeletal metastasis. Nevertheless, the role of GPIIb α in tumor cell-induced platelet aggregation remains controversial, with some reports showing its positive contribution [27], and others described no impact of blocking GPIIb α antibodies [28]. Also, in experimental metastasis models using B16F10 melanoma cells, mice lacking GPIIb α developed a lower number of lung metastases than wild type mice [29]. In a striking contrast, functional inhibition of GPIIb α using monoclonal antibodies *in vivo* led to a strong increase in pulmonary metastasis [30]. The reason for these discrepancies remain to be established.

6. Conclusion

Due to their specific location in vascular sinusoids, MKs appear as tempting targets for blocking skeletal metastases because they

might be the first cells encountered by metastatic cancer cells at early steps of bone colonization. Although TPO blocks PC3-induced skeletal metastasis in mice, such a regimen is unlikely to be transferred to the clinic because of an immediate increase in the circulating platelet count that could favor interactions between tumor cells and platelets, thereby increasing the risk of venous thromboembolism [31]. Targeting platelets and/or MKs may help inhibiting cancer metastases to the bone. However, to achieve efficacy and safety, strategies to block platelets' prometastatic activity will need to maintain platelets' vital functions in hemostasis while increasing the number of MKs in the bone marrow preventing bone degradation and stimulating bone formation.

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