British Journal of Pharmacology

British Journal of Pharmacology (2018) **175** 3100–3110 3100

REVIEW ARTICLE

Platelets, autotaxin and lysophosphatidic acid signalling: win-win factors for cancer metastasis

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Received 11 March 2018; **Revised** 26 April 2018; **Accepted** 1 May 2018

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Platelets play a crucial role in the survival of metastatic cells in the blood circulation. The interaction of tumour cells with platelets leads to the production of plethoric factors among which our review will focus on lysophosphatidic acid (LPA), because platelets are the highest producers of this bioactive lysophospholipid in the organism. LPA promotes platelet aggregation, and blocking platelet function decreases LPA signalling and leads to inhibition of breast cancer cell metastasis. Autotaxin (ATX), a lysophospholipase D responsible for the basal concentration of LPA in blood, was detected in platelet α-granules. Functionally, active ATX is eventually released following tumour cell-induced platelet aggregation, thereby promoting metastasis. Megakaryocytes do not express ATX but respond to LPA stimulation. Whether LPA-primed megakaryocytes contribute to the recently reported negative action of megakaryocytes on cancer metastasis is not yet known. However, an understanding of the ATX/LPA signalling pathways in platelets, cancer cells and megakaryocytes opens up new approaches for fighting cancer metastasis.

Abbreviations

ATX, autotaxin; LPA, lysophosphatidic acid; LPC, lysophospatidylcholine; NSAIDs, nonstreroidal anti-inflammatory drugs; TCIPA, tumour cell induced platelet aggregation; TEP, tumour-educated platelets; TPO, thrombopoietin

DOI:10.1111/bph.14362

Introduction

Platelets are well known allies of cancer cells during their transit in the blood circulation, supporting their survival in flux and successful implantation in secondary sites (Gay and Felding-Habermann, 2011; Leblanc and Peyruchaud, 2016). Over the past 40 years, the involvement of platelets in this process has been firmly demonstrated, and many essential pro-tumoural and pro-metastatic platelet-derived factors have been discovered and characterized (Gay and Felding-Habermann, 2011). A series of large randomized trials of daily aspirin (≥75 mg daily) versus control for the prevention of vascular events in the United Kingdom were used for evaluating the frequency of distant metastasis in patients who developed cancer during these trials (Rothwell *et al.*, 2012). This meta-analysis revealed that under such regimens, aspirin prevents distant metastasis, providing the support that including aspirin in cancer therapies might be beneficial in some cancers because of its preventative effect on distant metastasis (Rothwell *et al.*, 2012).

Whether such a characteristic of aspirin is shared with other nonsteroidal anti-inflammatory drugs (NSAIDs) remains to be determined. Aspirin inhibits **COX-1** and **COX-2** and consequently the production of inflammatory molecules (prostanoids) like other NSAIDs. This was considered to be the essential anti-tumoural mechanism of aspirin, through which it blocks the self-perpetuating inflammatory process during tumour growth (Zha *et al.*, 2004). More specifically, aspirin has a unique capacity to induce COX-2 acetylation, which increases the accumulation of potent anti-inflammatory molecules such as specialized proresolving lipid mediators derived from n-3 (ω-3) fatty acids or from a series of 15-epimers of lipoxin, **LXA_4**, known as **aspirin-triggered lipoxins** (Goh *et al.*, 2003; Barden *et al.*, 2015). However, the functional involvement of these molecules in the anti-tumoural action of aspirin requires experimental proof.

Despite this knowledge, targeting platelets has not yet been established as a standard of care for preventing metastasis in cancer patients. This is likely due to the vital role of platelets in haemostasis. Long-term depletion of platelet function may lead to an elevated risk of haemorrhage. This is one of the reasons why our laboratory and others are interested in discovering new therapeutic targets, with the perspective of blocking the pro-tumoural activity of blood platelets while not interfering with their physiological functions in haemostasis. Included among recently identified targets that may fall into this category are the interactions between podoplanin and C-type lectin-like receptor 2 (CLC2), and high-mobility group box 1 (HMGB1) with **toll-like receptor 4** (TLR4), whose functions have recently been addressed (Leblanc and Peyruchaud, 2016; Menter *et al.*, 2017). Our present review focuses on the intimate connections established between cancer cells and platelets involving the lysophosphatidic acid (**LPA**)/autotaxin (**ATX**) signalling pathways during the metastasis process.

LPA: sources and cancer involvements

LPA is the simplest natural lysophospholipid (Figure 1). Platelets were originally defined as major sources of LPA in the organism since LPA concentrations in plasma increase more than 10-fold in serum (from ~0.1 to >1 μM) (Eichholtz *et al.*, 1993). The serum levels of LPA are determined by a mechanism that involves diverse phospholipase pathways (Aoki *et al.*, 2002). However, knockout animal studies revealed that ATX (ectonucleotide pyrophosphatase/PDE2), a secreted glycosylated enzyme also present in blood, is responsible for basal levels of LPA in plasma (van Meeteren *et al.*, 2006). Due to its unique lysophospholipase D activity, ATX catalyses the production of LPA from a series of lysophospholipid precursors, including lysophosphatidylcholine (**LPC**), which is the most abundant in plasma (~200 μM), and also from lysophosphatidylserine and lysophosphatidylethanolamine (Aoki *et al.*, 2002). The outer membrane and microvesicles released from platelets were also found as sources of LPA downstream of the action of secreted **PLA_2** (Fourcade *et al.*, 1995). Mildly oxidized low-density lipoproteins are additional sources of LPA in the context of atherosclerosis (Siess and Tigyi, 2004).

LPA activates six different GPCRs (**$LPA_{1\text{-}6}$ receptors**) (Mutoh *et al.*, 2012) (Figure 1). Most eukaryotic cells express various combinations of LPA receptors that share multiple intracellular signalling pathways dependent on heterotrimeric G-proteins, including $G\alpha_i$, $G\alpha_{12/13}$, $G\alpha_q$ and $G\alpha_s$ (Noguchi *et al.*, 2009). Therefore, the pleiotropic activities of LPA (i.e. induction of cell survival, proliferation, cytoskeleton rearrangement, motility, cytokine secretion, cell differentiation) are likely the consequences of complex co-activation signals from multiple LPA receptor signalling pathways resulting in either redundant or opposite effects. This implies that in the context of developing efficient therapies using specific antagonists, potential LPA receptor redundancy should be carefully considered according to specificity of the pathological situation.

The role of LPA in cancer emerged in the late 1990s because of its aberrant production in several cancers, including ovarian and prostate cancers (Mills and Moolenaar, 2003). The expression of LPA receptors and LPA-dependent signalling pathways is altered in certain cancerous contexts. In colon cancer, specific inactivation of **LPA_2** or **LPA_3** receptors markedly affects LPA-induced cell proliferation dependent on the β-catenin pathway, suggesting a particular role for these receptors in the growth of intestinal tumours (Yang *et al.*, 2005). In contrast to the hormone-insensitive PC3 prostate cancer cells that express **LPA_1** receptors, the hormone-sensitive LnCap cells do not express this receptor and are incapable of generating xenograph tumours in mice unless co-injected with fibroblasts. Remarkably, *de novo* expression of LPA_1 receptor in LnCap cells confers self-autonomous growth and progression of xenograph tumours, suggesting that LPA_1 receptor expression may contribute to the hormonal switch during the progression of prostate cancers (Guo *et al.*, 2006). In the context of ovarian cancers, the expression levels of the EDG LPA receptors (LPA_2 and LPA_3 receptors) may function as rheostats. Overexpression and down-regulation strategies on SKOV-3 and OVCAR-3 cells showed direct correlations both *in vitro* to cell proliferation, migration and cytokine secretions and *in vivo* to tumour xenograph growth and metastasis. These receptors may play a critical role in the carcinogenesis and aggressiveness of ovarian cancer (Yu

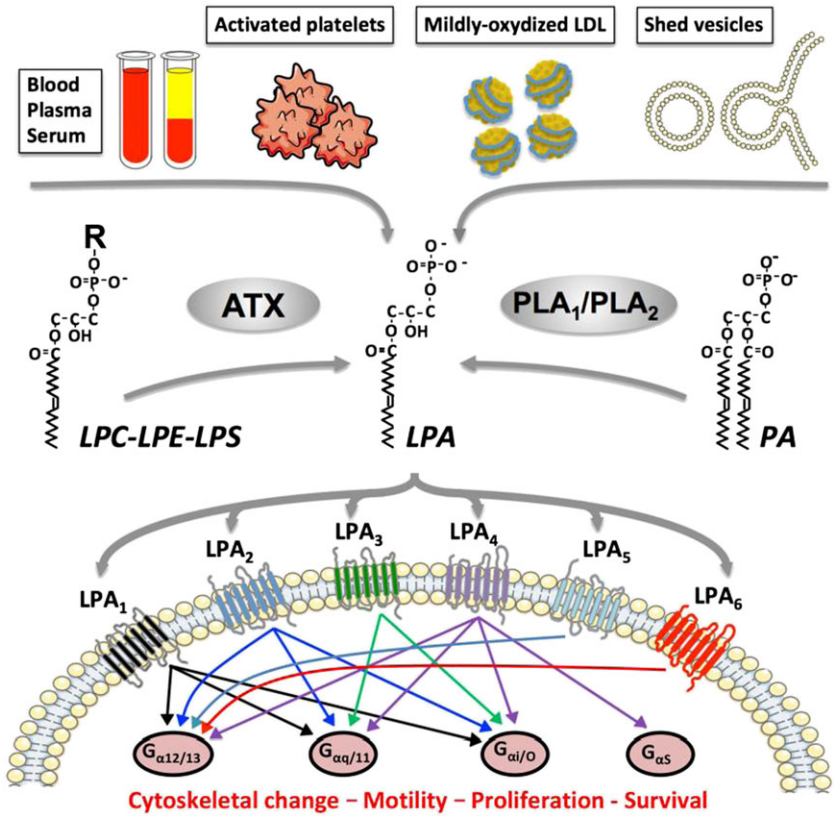

Figure 1

Summary of LPA origins and signalling pathways activated by six LPA receptors. R, choline, serine or ethanolamine; LPS: lysophosphatidylserine; LPE: lysophosphatidylethanolamine; PA, phosphatidic acid; G, heterotrimeric GTPase.

et al., 2008). MMTV-driven overexpression of ATX or each LPA receptor (LPA_1, LPA_2 and LPA_3 receptors) in mammary epithelium of transgenic mice was sufficient to induce carcinogenesis and metastasis of mammary cancer, demonstrating that ATX and LPA receptors can contribute to the carcinogenesis and progression of breast cancers (Liu *et al.*, 2009).

The concentrations of LPA and ATX are remarkably elevated in the context of nonmalignant inflammation and fibrosis where the LPA_1 receptor plays a central role in disease pathogenesis (Bourgoin and Zhao, 2010). Therefore, efforts were made to develop pharmacological molecules to target the LPA_1 receptor and/or ATX that were validated in preclinical animal models of fibrosis (idiopathic pulmonary fibrosis, dermal fibrosis, kidney fibrosis, systemic sclerosis) and inflammation (rheumatoid arthritis, asthma) (Table 1). An update on the IC_{50} values and specificities of a large series of LPA receptor blockers developed by the University of Memphis and different pharmaceutical companies was recently reviewed in detail by Llona-Minguez and co-workers (2015). Inflammation and fibrosis are also prevalent during cancer progression, supporting the role of LPA and ATX as important mediators in the tumour micro-environment (Benesch *et al.*, 2017). The efficacy of pharmacological drugs targeting LPA receptors and/or ATX has already been validated in multiple preclinical animal models of primary tumour growth and metastasis (Table 1).

Effect of LPA on platelets and megakaryocytes

The bioactive fraction of LPA in the circulation is bound to albumin and gelsolin (Meerschaert *et al.*, 1998). This may have an important impact on its bioavailability and capacity to activate specific receptors (Goetzl *et al.*, 2000). LPA concentration in blood is tightly buffered, which involves highly dynamic processes of formation/degradation (Morris *et al.*, 2009). However, the biological significance and pathophysiological roles of circulating LPA are still largely unrecognised. Circulating LPA might control different biological functions depending on animal species; it may control blood pressure as an i.v. injection of synthetic LPA causes hypertension in rats and guinea pigs but hypotension in cats and rabbits (Tokumura *et al.*, 1978).

LPA may also control platelet aggregation since it acts directly as a mild agonist on the aggregation of human platelets. In platelets from rabbits and dogs, LPA enhances **ADP**-induced aggregation, and after these platelets had been primed with a low dose of ADP, LPA is itself effective in stimulating aggregation (Gerrard *et al.*, 1979). In striking contrast, in murine platelets, LPA inhibits agonist-induced activation of these platelets (Pamuklar *et al.*, 2009). These remarkable functional differences dependent on animal species should be carefully taken into account when addressing

Table 1

List of pharmacological molecules targeting autotaxin and LPA receptors validated in preclinical animal models

Targets	Drug names	Diseases	References
LPA_1 receptor	AM966	Idiopathic pulmonary fibrosis	Swaney *et al.* (2010)
LPA_1 receptor	AM095	Dermal fibrosis	Castelino *et al.* (2011)
		Lung fibrosis	Swaney *et al.* (2011)
		Kidney fibrosis	Swaney *et al.* (2011)
LPA_1 receptor	LA-01	Rheumatoid arthritis	Miyabe *et al.* (2013)
LPA_2 receptor	DBIBB	Asthma	Knowlden *et al.* (2016)
LPA_2 receptor	GRI977143	Resistance to radiation	Kiss *et al.* (2013)
LPA_5 receptor	H2L-5765411	Thrombosis	Williams *et al.* (2009)
LPA_1, LPA_3 receptors	VPC-12249	Renal ischaemia–reperfusion	Okusa *et al.* (2003)
LPA_1, LPA_3 receptors	Ki16425	Rheumatoid arthritis	Orosa *et al.* (2014)
		Hydrocephalus	Yung *et al.* (2011)
		Renal interstitial fibrosis	Pradere *et al.* (2007)
		Cancer:	
		Breast cancer bone metastasis	Boucharaba *et al.* (2004)
		Renal cell carcinoma	Su *et al.* (2013)
LPA_1, LPA_3 receptors	Debio 0719	Osteoporosis	David *et al.* (2014)
		Cancer:	
		Breast cancer liver metastasis	Marshall *et al.* (2012)
		Breast cancer lung metastasis	Marshall *et al.* (2012) and David *et al.* (2012)
		Breast cancer bone metastasis	David *et al.* (2012)
LPA_1, LPA_3 receptors	Ki16198	***Cancer***:	
		Pancreatic cancer	Komachi *et al.* (2012)
		Pancreatic cancer lung, liver and brain metastases	Komachi *et al.* (2012)
All LPA receptors and autotaxin	BrP-LPA	Rheumatoid arthritis	Nikitopoulou *et al.* (2013)
		Cancer:	
		Breast cancer	Zhang *et al.* (2009)
		Glioma	Schleicher *et al.* (2011)
Autotaxin	VPC8a202	***Cancer***:	
		Breast cancer lung metastasis	Peyruchaud *et al.* (2013)
Autotaxin	ONO-8430506	***Cancer***:	
		Breast cancer	Benesch *et al.* (2014)
		Breast cancer lung metastasis	Benesch *et al.* (2014)
Autotaxin	PF-8380	Inflammation	Gierse *et al.* (2010)
		Cancer:	
		Glioblastoma	Bhave *et al.* (2013)
Autotaxin	4PBPA	***Cancer***:	
		Melanoma lung metastasis	Gupte *et al.* (2011)
Autotaxin	S32826	Glaucoma	Iyer *et al.* (2012)
Autotaxin	BMP-22	***Cancer:***	
		Breast cancer bone metastasis	Leblanc *et al.* (2014)
Autotaxin	Gintonin	***Cancer:***	
		Melanoma lung metastasis	Hwang *et al.* (2013)
Autotaxin	GWJ-A-23	Idiopathic pulmonary fibrosis	Oikonomou *et al.* (2012)
		Asthma	Park *et al.* (2013)

the biological functions of LPA, at least with regard to its contribution as a systemic factor in blood and more specifically on platelet functions.

Among the healthy population, platelets obtained from 20% of human donors fail to aggregate in response to LPA (Pamuklar *et al.*, 2008). Since the first identification of the existence of a cell surface LPA receptor based on platelet studies (Watson *et al.*, 1985) and cloning of Vzg1/Edg-2 as the first LPA receptor (known now as LPA_1 receptor) (Hecht *et al.*, 1996), all six LPA receptor mRNAs (LPA_{1-6} receptors) have been found in human platelets (Rowley *et al.*, 2011). Interestingly, an overexpression of **LPA_4** was detected in platelets from unresponsive patients (Pamuklar *et al.*, 2008). LPA_4 has the unique ability to link to $G\alpha_S$, resulting in the activation of **adenylate cyclase** and consequently causing an increase in **cAMP** levels in cells; whereas other LPA receptors activate Gα that at the opposite end inhibits adenylate cyclase (Figure 1). An increase in intracellular levels of cAMP inhibits platelet aggregation (Noe *et al.*, 2010). Therefore, the unresponsiveness of human platelets to LPA is likely the consequence of predominant activation of the $G\alpha_s$ rather than the $G\alpha_i$ pathways promoting adenylate cyclase activation and increasing cAMP levels. As judged by RNA-seq analyses, the repertoire of LPA receptor mRNAs differs between human and murine platelets. *LPAR5* mRNA is remarkably prevalent in human platelets but totally absent in murine platelets (Rowley *et al.*, 2011). The **LPA_5** receptor has unique ligand selectivity to alkyl-LPAs, whereas acyl-LPAs are the most common ligands for all other LPA receptors. Intriguingly, alkyl-LPAs revealed higher potencies than acyl-LPAs in activating platelets (Tokumura *et al.*, 2002). Moreover, silencing, individually, each LPA receptor in Meg-01 and Dami megakaryocyte cell lines revealed that only LPA_5 receptor knockdown significantly inhibited LPA-induced shape change (Khandoga *et al.*, 2011). These results provide strong evidence that the LPA_5 receptor is the functionally active LPA receptor in platelets (Williams *et al.*, 2009) (Figure 2).

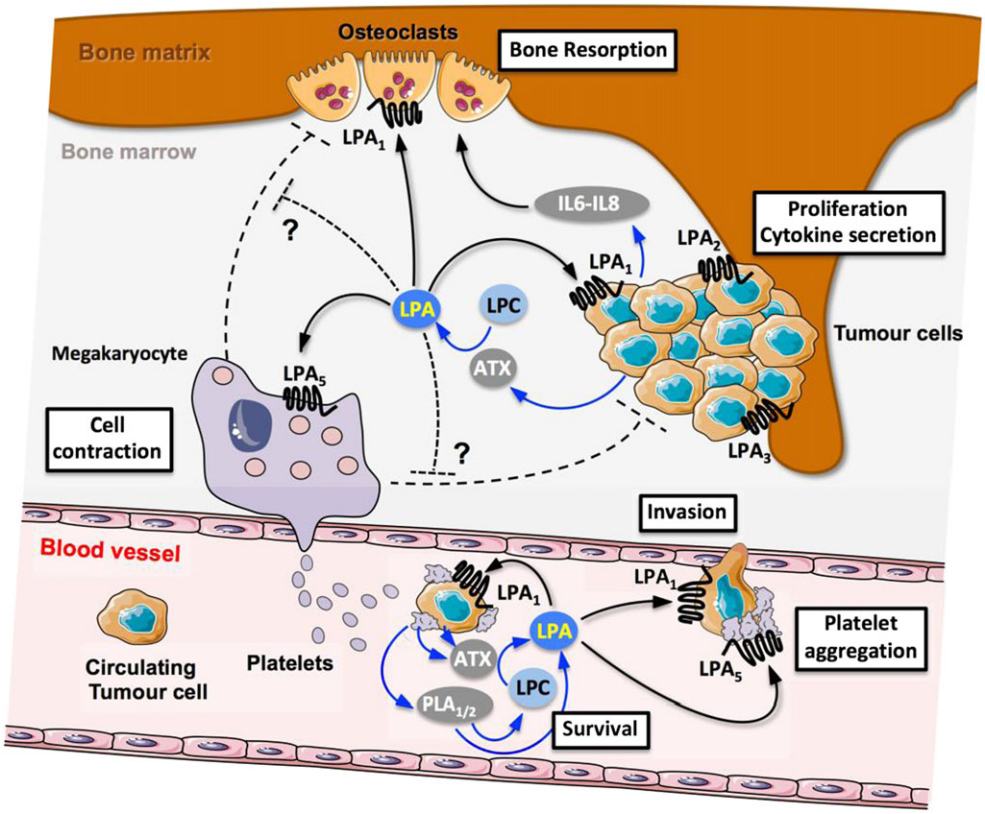

Figure 2

Summary of coupling actions between blood platelets, ATX, LPA and LPA receptors in bone metastasis. Interaction of circulating tumour cells with platelets induces platelet aggregation and release of LPA through mechanisms involving phospholipases A1 and A2 ($PLA_{1/2}$) generating LPA directly or indirectly through synthesis of LPA precursors including LPC that was eventually degraded by ATX mobilized from the blood circulation or secreted by platelets. In the blood circulation, LPA will act on tumoural LPA_1 receptors promoting survival and invasion and potentially on platelet LPA_5 receptors promoting platelet aggregation. After colonizing the bone marrow, LPA will act on tumoural LPA_1 receptors promoting cell proliferation and pro-osteoclastic cytokine (IL-6, IL-8) secretion promoting bone resorption indirectly or directly by acting on osteoclast LPA_1 receptors. LPA might act on megakaryocyte LPA_5 receptors to induce cell contraction. It is proposed that the pro-metastatic action of LPA in bone metastasis might involve the action of LPA on megakaryocytes counteracting their negative action on osteoclast and tumour cells, blunting their protective action against bone metastasis progression. ?, requires experimental validation.

Bone metastases: paramount sites involving LPA, platelets and megakaryocytes

Pharmacologically- or genetically-induced thrombocytopenia dramatically affects the capacity of cancer cells to seed to distant sites (Gasic *et al.*, 1968; Boucharaba *et al.*, 2004; Camerer *et al.*, 2004). Functional blocking of platelet activity also alters metastasis; combined treatments of mice with ATP102, a soluble ADPase, and aspirin were shown to significantly reduce melanoma and breast cancer bone metastasis (Uluckan *et al.*, 2008). We showed that thrombocytopenia induced in mice treated with integrelin leads to reduced levels of circulating LPA and to a remarkable decrease in LPA-dependent growth of skeletal tumours. Thus, at the bone site, platelet activity could generate bioactive LPA that may act on cancer cells promoting tumour progression and metastasis (Boucharaba *et al.*, 2004) (Figure 2).

Metastatic cancer cells have the ability to interact with platelets and inducing platelet aggregation through mechanisms involving β3 integrins and P-selectin among others (Borsig, 2008). Human MDA-MB-231 and MDA-B02 breast cells display these characteristics; but beyond physical interaction with platelets, tumour cell-induced platelet aggregation (TCIPA) mediates the production of multiple factors including LPA (Boucharaba *et al.*, 2004). Remarkably, these cancer cell lines do not express ATX and are incapable of synthesizing LPA, indicating that LPA produced during TCIPA is derived directly from aggregated platelets. Then, LPA can act as a paracrine factor on tumour cells *via* the LPA_1 receptor, promoting cell proliferation, migration and secretion of pro-inflammatory and pro-osteoclastic cytokines and growth factors [**IL-6**, **IL-8**, **granulocyte-macrophage colony stimulating factor** (GM-CSF), **chemokine ligand 2** (CCL2)]. These tumour cell-derived factors are major contributors to the effects of platelets on the progression of osteolytic bone metastases (Boucharaba *et al.*, 2004; Boucharaba *et al.*, 2006) (Figure 2).

However, the murine models used in these studies are not relevant for addressing LPA-mediated crosstalk between platelets and cancer cells. As demonstrated previously, in contrast to those of humans, murine platelets do not respond to LPA. In humans, LPA produced during TCIPA could potentially act as an autocrine factor on platelets through LPA_5 receptors, which could further enhance platelet aggregation and increase tumour metastasis (Figure 2). As a consequence, the LPA_5 receptor appears to be an attractive target for the development of new therapies against the metastatic effects of platelets. However, pharmacological investigations into this approach would require animal subjects with competent platelets that respond to LPA stimulation, such as those from dogs or guinea pigs.

Transgenic mice overexpressing **thrombopoietin** (TPO) exhibit an increased number of megakaryocytes in the bone marrow and develop a high bone mass phenotype (Yan *et al.*, 1996). The increased expansion of megakaryocytes in mice in response to a 5-day TPO administration prior to intracardiac injection of PC3 prostate cancer cells remarkably decreases the extent of skeletal lesions and tumour burden (Li *et al.*, 2011). TPO was shown to inhibit osteoclast differentiation and their resorption activity *in vitro* (Wakikawa *et al.*, 1997). Blocking osteoclast function with anti-resorptive agents is the current care of patients with hypercalcaemia and bone metastases. Thus, TPO might prevent osteolysis directly and indirectly through increased production of megakaryocyte-derived osteoclast inhibitors (Wakikawa *et al.*, 1997). In addition, murine primary megakaryocytes inhibit the proliferation and increase the apoptosis of prostate cancer cells in co-culture systems, and this may contribute to the inhibition of skeletal tumour growth (Figure 2). These results support recent clinical findings that increased levels of circulating megakaryocytes tend to correlate with good prognosis in patients with prostate cancer and that a combination of circulating tumour cell levels and megakaryocyte count may predict survival in advanced case of this disease (Xu *et al.*, 2017).

Megakaryocytes are located adjacent to bone marrow blood vessels allowing plasma membrane expansion through the endothelium and production of platelets (Kaushansky, 2008) (Figure 2). Megakaryocytes express a large series of growth factors including **PDGF-β** and **FGF-2**, which promote osteoblast differentiation and may help to maintain a high bone mass (Kacena *et al.*, 2004). Megakaryocytes also express functionally active anti-angiogenic factors such as thrombospondins 1 and 2 (**TSP1** and TSP2), as seen from Matrigel plugs loaded with double KO-TSP1/TSP2 megakaryocytes that had reduced sprouting vessels compared to that with wild-type megakaryocytes (Kopp *et al.*, 2006). These findings suggest that megakaryocytes potentially control osteoblastic and haematopoietic vascular niches that are functionally important anatomical structures for the successful establishment of bone metastasis (Psaila and Lyden, 2009). Until recently, opposite theories were proposed for the impact of megakaryocytes on these niches in promoting or inhibiting metastasis (Zaslavsky *et al.*, 2006; Li *et al.*, 2011; Psaila *et al.*, 2012). However, in 2017, using preclinical models of breast cancer metastasis, Jackson and colleagues (2017) demonstrated that an increase in the number of megakaryocytes occurs in response to metastatic cells entering the bone marrow. The molecular mechanisms involved in this process have not yet been elucidated. However, compared with wild-type mice, $TPO^{-/-}$ animals injected orthotopically with 4T1.2 metastatic murine carcinoma cells displayed more aggressive metastasis and a decreased survival. In this context, the presence of megakaryocytes may protect against skeletal metastasis (Figure 2). Both PC3 prostate cancer cells and 4T1 breast carcinoma cells express ATX and produce LPA autonomously, whereas megakaryocytes are incapable of this (Leblanc *et al.*, 2014). The contribution of the different-shaped megakaryocytes induced by LPA, through the LPA_5 receptor, to a host's reaction to metastasis is totally elusive but would deserve further investigation (Figure 2).

Functional interaction of ATX with platelets and cancer cells

ATX is a multidomain protein that possesses two somatomedin B (SMB1,2)-like domains: a catalytic PDE domain and a nuclease-like domain. A previous study on the role of ATX/LPA in murine thrombosis has shown that activated

but not resting platelets are able to bind recombinant ATX. This binding process can be disrupted by 7E3 antibody, demonstrating the involvement of β3 integrin (Pamuklar *et al*., 2009). Although ATX possesses the classical RGD motif that mediates integrin binding, the recent crystal structure reveals that ATX-SMB2 might interact with platelet-**αIIbβ3** integrins using a surface similar to that used by the related vitronectin SMB domains in their interactions with plasminogen activator inhibitor-1 (PAI-1) and a urokinase receptor (uPAR; Hausmann *et al*., 2011). Fulkerson *et al*. also investigated the integrin signalling pathways that promote ATX binding to platelets. They found that ATX increases thrombin-stimulated LPA production by washed platelets and provided evidence that ATX-mediated LPA production is significantly higher in CHO cells transfected to express αIIbβ3 integrin. Moreover, blocking the ATX/αIIbβ3 interaction by performing point mutations in the SMB2 domain or using 7E3 antibody leads to a decrease in LPA production (Fulkerson *et al*., 2011).

Although ATX expression is elevated in several types of cancers (neuroblastoma, beta cell lymphoma, melanoma, breast carcinomas, etc.) and correlated with a poor prognosis (Leblanc and Peyruchaud, 2015), it is now well accepted that stromal cells including platelets can provide ATX to the tumour for enhancing cancer progression (Figure 2). Based on immunohistochemistry analysis of murine breast carcinoma tissue, Benesch and colleagues (2014) noticed a higher ATX staining in the stroma than in the tumour cell compartment. In addition, adipose tissue that has a remarkable impact on breast cancer is also known to be a major source of ATX in the organism (Dusaulcy *et al*., 2011). Recently, Brindley's lab revealed that tumour-induced inflammation in mammary adipose tissue stimulates a vicious cycle of ATX expression and breast cancer progression (Benesch *et al*., 2015).

By using a human breast cancer cell line (MDA-B02) that does not express ATX, we also found that treatment of animals with an ATX inhibitor (BMP22) inhibited both the progression of pre-established skeletal metastases and the early steps of cancer cell colonization to the bone (Leblanc *et al*., 2014) (Figure 2). Furthermore, we demonstrated for the first time that ATX can be stored in platelet α-granules isolated from healthy donors and released upon TCIPA, leading to the production of LPA *via* the degradation of platelet-derived LPC (Leblanc *et al*., 2014). We showed that the pro-tumoural activity of ATX derived from platelets was partially dependent on the interaction of ATX with tumoural **αvβ3 integrin**. A recent report also demonstrated a cooperative action between exogenous ATX and β3 integrin in cell migration. The binding of ATX to integrin enabled the uptake and redistribution of ATX to the leading edge of migrating cells (Wu *et al*., 2014). Altogether, these studies suggest that β3 integrin binding might localize ATX activity to the cancer cell/platelet surface, providing a mechanism to generate LPA in the vicinity of its receptors, and then enhance cancer cell dissemination.

There is extensive experimental evidence indicating that platelets also support cancer cell extravasation to secondary metastatic sites (Labelle *et al*., 2011) (Figure 2). For instance, dual blocking of the platelet-membrane P-selectin and **integrin αIIb/IIIa** reduced the ability of tumour cells to degrade Matrigel, confirming their role in assisting tumour cells in the extravasation process (Pang *et al*., 2015). Our recent publication also suggests that by increasing the ability of the MDA-MB-231 cell line to cross an endothelial monolayer and to degrade a Matrigel layer, LPA and its precursor ATX may favour breast cancer cell extravasation to the bone (Leblanc *et al*., 2014). Kanda and colleagues (2008) have already described such a mechanism, where they showed that ATX binds to chemokine-activated human lymphocytes in a **β1 integrin**-dependent manner. This binding promotes LPA production and enhances the entry of lymphocytes into secondary lymphoid organs. Interestingly, Smyth's group reported that platelet β1 integrin also interacts in an activation-dependent manner with ATX *via* the SMB2 domain (Fulkerson *et al*., 2011). Since activated platelets can produce and bind ATX, as well as participate in lymphocyte trafficking in high endothelial venules (Diacovo *et al*., 1996), we could imagine platelet β1 integrin cooperates with ATX in the tumour cell extravasation process.

Platelets: a function beyond aggregation

Platelets are fascinating cells. They do not have a nucleus and therefore they lack gene transcription. Nevertheless, platelets benefit from the large amount of plethoric factors (proteins, lipids, nucleotides) produced by megakaryocytes that in turn are responsible for the role of platelets in haemostasis, cancer and other pathologies. Platelets also contain residual mRNAs synthesized by megakaryocytes that are efficiently used as templates for *de novo* protein synthesis (Rowley and Weyrich, 2013). Specific platelet transcriptional profiles and protein expressions have not yet been determined in the context of cancer, but they have been confirmed in patients with several types of diseases including cardiovascular disease, sickle cell anaemia and systemic lupus erythematosus (Rowley *et al*., 2012).

MicroRNAs are epigenetic factors controlling gene expression through indirect destabilization of mRNAs. They are also present and functionally active in platelets, as shown from platelet factor 4-Cre-mediated deletion of Dicer1 that heightened platelet reactivity (Rowley *et al*., 2016). In addition to the presence of factors derived from megakaryocytes, platelets have also a remarkable capacity to take up multiple factors from the surrounding environment during their short life of 9–12 days in the blood stream. This is well known for fibrinogen, albumin and immunoglobulin (Handagama *et al*., 1990). We recently demonstrated that this is also the case for ATX since it is not synthesized by megakaryocytes but found in platelet α-granules and released upon TCIPA (Leblanc *et al*., 2014). This suggests that in the perspective of new therapeutics directed against ATX, stored protein in α-granules is unlikely to be accessible to pharmacological drugs and would escape inactivation leading to potentially ineffective treatments.

Since platelets are circulating throughout the entire organism, they may also be able to collect release factors related to specific pathological situations appearing in different organs. In an oncological context, Best and colleagues (2015) recently demonstrated that tumour-educated platelets (TEP) could be used as a liquid biopsy that, following up RNA-Seq

analysis, leads them to distinguish patients with localized and metastasized tumours from healthy individuals. Subsequent algorithm optimization of TEP analysis allowed this group to detect early- and late-stage non-small cell lung cancer (Best *et al.*, 2017).

Conclusion

Beside their vital role for human health, platelets also promote cancer progression and metastasis. In this context, platelets can be used as therapeutic targets and prognostic tools. The complexity of the ATX/LPA signalling axis in cancer has recently reached a higher level since both platelets and several tumour cells were found to produce LPA, thereby promoting platelet aggregation and tumour cell proliferation and mobility. As a consequence, platelets and cancer cells participate in a vicious cycle whereby tumour cell proliferation and platelet aggregation sustain each other. In addition, ATX-null cells that would be deficient in LPA signalling could benefit from platelet-derived ATX for eliciting LPA-induced programmes leading to cancer metastasis. Once established into secondary sites, metastatic cells acquire resistance to conventional treatments. Understanding how circulating tumour cells survive in flux before seeding in target organs remains crucial for the development of potent anti-metastasis therapies.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b,c).

Acknowledgements

Figures were produced using Servier Medical Art (http://www.servier.com). This work was supported by grants from INSERM (O.P.) and the Université Claude Bernard Lyon-1 (O.P.), the Comité Départemental de la Savoie de la Ligue Contre le Cancer (O.P.), the Fondation ARC pour la Rechercher sur le Cancer (ARC, grant no. PJA20151203151) and the ANR grant LYSBONE (O.P.) (grant no. ANR-15-CE14-0010-01). R.L. is a recipient of a grant fellowship from the Fondation ARC pour la Recherche sur le Cancer.

Conflict of interest

The authors declare no conflicts of interest.

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