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Review Article

Autotaxin: Its Role in Biology of Melanoma Cells and as a Pharmacological Target

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Autotaxin (ATX) is an extracellular lysophospholipase D (lysoPLD) released from normal cells and cancer cells. Activity of ATX is detected in various biological fluids. The lysophosphatidic acid (LPA) is the main product of ATX. LPA acting through specific G protein-coupled receptors (LPA_1-LPA_6) affects immunological response, normal development, and malignant tumors' formation and progression. In this review, the impact of autotoxin on biology of melanoma cells and potential treatment is discussed.

1. Biochemistry and Structure of Autotoxin

Autotaxin, ATX (E.C. 3.1.4.39), is a member of the family of nucleotide pyrophosphatases/phosphodiesterase (NPP1-7) and is also referred, as NPP2 [1]. It is to a glycoprotein with four possible N-glycosylation sites, synthesized as a pre-proenzyme and is secreted to extracellular space following two N-terminal cleavages (27 and 8 amino acids) [2, 3]. ATX is a constitutively active enzyme possessing activity of phospholipase D. It hydrolyzes the head groups of lysophospholipids to lysophosphatidic acid (1 or 2-acyl-*sn*-glycerol-3-phosphate, LPA) and also acts on sphingosylphosphorylcholine to produce sphingosine 1-phosphate (S1P) [4, 5]. Both, LPA and S1P are strong inhibitors of ATX with affinity to enzyme approximately 1000-fold higher than reported for ATX substrates [6]. mRNA for ATX has been detected in brain, ovary, lung intestine, and kidney but enzyme activity has been detected in blood, cerebrospinal and seminal fluid, urine, and saliva [7–11]. It is not filtered in the glomerulus because of high molecular weight (~125 kDa) but is cleared from the circulation by the scavenger receptors of liver sinusoidal endothelial cells [12]. Moreover, ATX is the main source of blood LPA (~0.1 μM plasma and ~1 μM serum), however, not for S1P [13, 14]. There is evidence that S1P is produced intracellularly *via* sphingosine kinases and transported through ATP-binding cassette transporter [15, 16]. ATX hydrolyzes also ATP; however, affinity to ATP is at least 50-fold lower then for lysophospholipids [17, 18]. LPA acts on target cells through specific G-protein-coupled receptors: LPA_1/Edg2, LPA_2/Edg4, LPA_3/Edg7, LPA_4/GPR23/$P2Y_9$, LPA_5/GPR92, LPA_6/$P2Y_5$ broadly expressed in normal and cancer cells [19, 20]. The LPA-binding proteins for example, albumin, determine the activation of the specific LPA receptors; LPA_3, in contrast to LPA_1 and LPA_2, is not activated by complex LPA-albumin [21].

The structure of autotoxin is presented in Figure 1. At the N-terminus, ATX possesses hydrophobic signal sequence (SS) followed by two somatomedin B-like domains (SBLD) with RGD tripeptide motif suggesting that this domain may play a role in cell-extracellular matrix interactions. At catalytic domain (CD), Thr^{210} and N542-linked glycan are suggested to be responsible for hydrolytic activity of ATX, but motif G/FXGXXG is responsible for metal binding. ATX activity is stimulated by divalent cations for example, Ca^{2+}, Mg^{2+}, and Co^{2+}. C-terminally to CD is the nuclease-like domain (NLD). It contains EF-hand-like motif and is structurally similar to DNA and RNA-nonspecific endonucleases; however, it lacks the catalytic activity. In humans, NLD is covalently linked with catalytic domain *via* disulfide

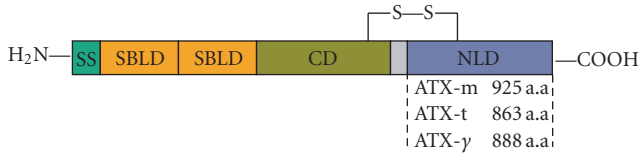

Figure 1: Scheme of the structure domains of autotaxin isoforms. SS: signal sequence, SBLD: somatomedin B-like domains, CD: catalytic domain, NLD: nuclease-like domain.

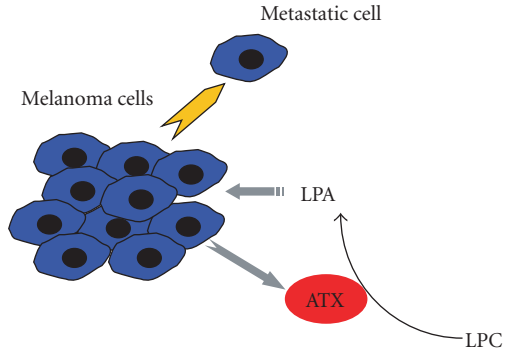

Figure 2: Role of ATX-LPA axis in motility of melanoma cells. ATX: autotaxin, LPA: lysophosphatidic acid, and LPC: lysophospholipids.

bridge (C413-C805). The ATX gene is located on chromosome 8 at position 8q24.1 and ATX has three alternative splicing isoforms in humans: ATX teratocarcinoma-derived ATX-t (925 a.a); melanoma-derived ATX-m (863 a.a), and brain specific, ATX-γ (888 a.a) [22–25].

2. Role of Autotoxin during Normal Development

ATX has a critical role in formation of vasculature by vasculogenesis and angiogenesis. ATX knockout mice ($atx^{-/-}$) are lethal around embryonic day 10.5. Admittedly, ATX is a major producing enzyme for LPA, nevertheless the phenotypes of LPA receptors knockout mice is less severe, suggesting that ATX-induced cellular signal may involve others pathways. This speculation is supported by results of experiments where modification of LPA level in blood (2-fold increase) by driving ATX expression is not sufficient to induce tumorigenesis [13, 26–28].

3. Role of Autotoxin in Biology of Melanoma Cells

ATX was identified in the cultured cell supernatant of human melanoma cells (A2058) as a cell motility-stimulating factor acting at pM-nM concentrations in pertussis toxin-sensitive manner [29]. Further studies have provided evidence that LPA, product of ATX, mediates chemotaxis and proliferation of melanoma cells [30]. The recent experiments suggest that ATX expression is one of the factors involved in metastasis of melanoma cells (Figure 2). Inhibition of ATX production blocks LPA-induced migration of melanoma cells [31]. It has been detected that melanoma metastatic specimens have increased ATX level, and ATX expression in primary melanoma is higher than in melanoma *in situ* [32]. Moreover, reduced expression of ATX predicts survival in uveal melanoma [33].

It has been shown that ATX-stimulated motility is suppressed by an LPA_1-selective antagonist, Ki16425, in melanoma cells [34]. Accumulating evidence suggest the various intracellular signaling pathways may be involved in ATX-induced motility of melanoma cell. It has been shown that this action is mediated through G-protein coupled isoform of phosphatidylinositol 3-kinase γ (PI3Kγ) suggesting involvement of proteins located downstream of PI3Kγ, for example, small G proteins [35]. Accordingly, there is evidence that ATX induces Cdc42/Rac1/p21-activated kinase (PAK1) complex formation [36]. The experimental data suggest that this complex is required for LPA-induced activation of focal adhesion kinase (FAK) [37]. The changes of PAK1 and FAK activity affect cytoskeleton proteins and structural integrity of melanoma cells. Moreover, ATX in melanoma cells induces the expression and activity of urokinase-type plasminogen activator (UPA) in a dose-dependent manner. This action is mediated by G_i proteins and PI3K/Akt signaling involving translocation of p65 into the nucleus and DNA binding of necrosis factor kappa B [38]. In general, action of LPA on melanoma cells enhances their metastaic potential (Figure 3). Recent experiments have provided evidence about the role of LPA receptors in biology of melanoma cells [32]. Downregulation of LPA_3 and using sequence-specific small interfering RNA (siRNA) reduces melanoma cells viability and proliferation. The effects of LPA receptors activation in melanoma cells are presented in Figure 4.

4. Autotoxin as a Pharmacological Target

The approved melanoma therapy lacks significant efficiency, hence, new therapeutic targets are under investigation. The current research focuses on the autotoxin-LPA axis [39]. Because LPA acts on multiple receptors with overlapping activities, currently the LPA receptors are not attractive as a pharmacological target, and the main stream of investigation concerns ATX activity.

4.1. L-Histidine. L-histidine inhibits activities of ATX in a non-competitive manner with $IC_{50} \sim 4$ mM and ATX-stimulated migration of human melanoma cells; 10 mM L-histidine induces 90%–95% reduction in stimulated motility. The proposed mechanism of L-histidine action is based on inhibition of a process that is required for the hydrolysis of both nucleotides and phospholipids [40].

4.2. Analogs of Bioactive Lipids. ATX is negatively regulated by LPA. The effect is dependent on the length of the acyl chain; maximal inhibition is induced by 1-oleoyl-LPA ($IC_{50} \sim 0.1$–$2\,\mu$M), 1-palmitoyl-LPA, and 1-myristoyl-LPA whereas short-chain LPA (6 : 0) has no measurable effect. The inhibition of ATX activity results from a combination

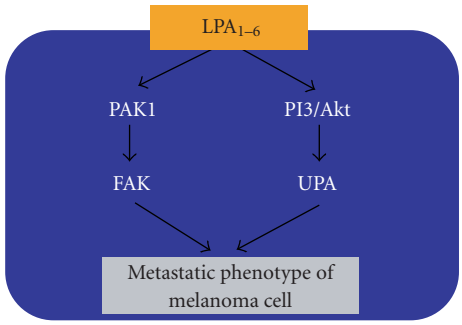

Figure 3: A proposal role for LPA receptors during melanoma progression. LPA receptors activation in melanoma cells leads to p21-activated kinase (PAK) and focal adhesion kinase (FAK) and to the activation of phosphoinositide 3-kinase/Akt-kinase and urokinase-type plasminogen activator (UPA). Finally, the melanoma cells express the metastatic phenotype.

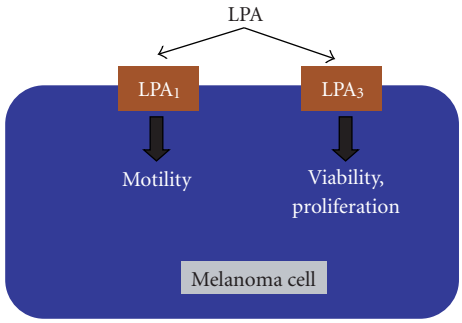

Figure 4: The LPA_1 and LPA_3 receptors as a potential target for pharmacological intervention.

of a decreased turnover number and decreased affinity of the active site for its substrates. Of note, LPA both inhibits ATX and activates LPA receptors (EC_{50} for LPA 18 : 1 at LPA_{1-4} ~ 0.007–0.5 μM), therefore it may induce the opposite of the intended effect [6, 41].

The other group of ATX inhibitors are analogues of cyclic phosphatic acid (1-acyl-*sn*-glycero-2,3-cyclic-phosphate, cPA) [42, 43]. The naturally occurring cPA, 1-oleoyl-*sn*-glycero-2,3-cyclic-phosphate (cPA 18 : 1) possesses unique properties having two targets: enzyme and receptor. cPA 18 : 1 inhibits ATX activity with a maximum of 22% at 1 μM and inhibits signaling pathway mediated through LPA_1 and LPA_3 receptors. Because of this properties, cPA 18 : 1 is described as having a "one-two punch" [44]. Another naturally occurring cPA, cPA 16 : 1 (1-palmitoleoyl-*sn*-glycero-2,3-cyclic-phosphate) possesses similar activity against ATX with maximum of 45% at 1 μM.

Interestingly, replacement of either the *sn*-2 or *sn*-3 oxygen by methylene (carba group) increases the inhibitory properties of these analogues (carba analogues, ccPA) [45]. 2ccPA 16 : 1 and 2cPA 18 : 1 (replacement of *sn*-2 oxygen with a methylene group) inhibit ATX activity about 90% with IC_{50} ~ 140 nM and ~370 nM, respectively. Furthermore, 3ccPA 16 : 1 and 3ccPA 18 : 1 (replacement of *sn*-3 oxygen with methylene group) inhibit ATX activity about 70% with IC_{50} ~ 300 nM and ~60 nM, respectively. Moreover, 3ccPA does not interact with LPA receptors. The invasion assay using melanoma cells (A2058) has provided evidence that ccPA-induced inhibition of ATX activity results in inhibition of cell migration. Furthermore, cPA and ccPA exert an inhibitory effect on experimental pulmonary metastasis in mice. Recently, a new generation of ccPA with potential therapeutic modality has been developed. Thio-ccPA 18 : 1 possesses multitarget properties. It inhibits ATX activity (~90% at 10 μM) and blocks LPA_1 (IC_{50} ~ 800 nM) and LPA_3 (IC_{50} ~ 440 nM) receptors without effect on LPA_2 receptors. Thio-ccPA influences metastatic melanoma tumors *in vivo*, reducing the number of pulmonary metastases and metastatic lesions to kidney, liver, pancreas, and intestines [32].

4.3. Nonlipid Small Molecule. It has been recently shown that thiazolidinediones compounds with incorporated boric acid moiety into catalic T210 residue (HA 130) inhibit ATX-mediated LPA production with IC_{50} ~ 30 nM [46]. Intravenous injection of HA 130 decreases 3.8-fold plasma LPA level in mice at 10 min. Furthermore, HA 130 inhibits ATX-mediated melanoma cells migration without affecting LPA receptor signaling pathways.

A report has been recently published describing the pharmacokinetic and pharmacodynamic properties of PF-8380 [47]. It inhibits activity of isolated ATX or ATX activity in blood with IC_{50} ~ 3 and 100 nM, respectively. There are no data about influence on melanoma cells, hoverer, PF-8380 (30 mg/kg) taken orally decreases the plasma LPA level about 95%, suggesting its potential usage in melanoma treatment.

There are several small-molecule, nonlipid ATX inhibitors including hexachlorophene, merbromin, bithionol, and others under investigation [48, 49]. Their mechanism of action differ (competitive, noncompetitive or mixed inhibition) and the most potent compounds inhibit ATX activity with IC_{50} at micromolar range. Their biological action was confirmed in experiments *in vivo* measuring effects on melanoma cell motility and invasion. A recently developed new TX autotaxin inhibitor pipemidic acid inhibits ATX with IC_{50} ~ 900 nM [50]. The natural phenolic antioxidants, including flavonols, possess inhibitory properties against ATX; however, the effect on ATX activity is about 2-fold lower than LPA 16 : 1 (1-palmitoleoyl-sn-glycerol-3-phosphate). Moreover, it has been estimated that it would be difficult to affect ATX activity *in vivo* by flavonoids supplementation in diet because plasma concentration of flavonoids in plasma may reach 10 μM [51].

The recently published crystallography results are used in ligand-based computational approaches for optimization of the current ATX inhibitors and development of new ones [52, 53].

Taken together, the increasing incidence of melanoma and poor average survival of metastatic melanoma are the main reason for the development of the new chemical compounds used in melanoma treatment. Autotaxin, melanoma cell motility-stimulating factor, and their receptors seem to be promising targets for pharmacological treatment

of melanoma. Much more research is needed for synthesis and pharmacological characterization of new specific ATX or LPA receptors inhibitors.

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