Review Article

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

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1. Biochemistry and Structure of Autotaxin

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 preproenzyme 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 (LPA1-LPA6) affects immunological response, normal development, and malignant tumors’ formation and progression. In this review, the impact of autotaxin on biology of melanoma cells and potential treatment is discussed.

The structure of autotaxin is presented in Figure 1. At the N-terminus, ATX possesses hydrophobic signal sequence 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), Thr210 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, Ca2+, Mg2+, and Co2+. 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 bonds.
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 LPA1-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 G1 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 metastatic potential (Figure 3). Recent experiments have provided evidence about the role of LPA receptors in biology of melanoma cells [32]. Downregulation of LPA3 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 IC50 ∼ 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 (IC50 ∼ 0.1–2 µM), 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...
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 (EC50 for LPA 18:1 at LPA1 = 4.007–0.5 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 LPA1 and LPA3 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 70% with IC50 ~ 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 LPA1 (IC50 ~ 800 nM) and LPA3 (IC50 ~ 440 nM) receptors without effect on LPA2 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 thiazolinediones compounds with incorporated boric acid moiety into catalic T210 residue (HA 130) inhibit ATX-mediated LPA production with IC50 ~ 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 IC50 ~ 3 and 100 nM, respectively. There are no data about influence on melanoma cells, however, 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 IC50 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 IC50 ~ 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-glycero-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.

References

[1] C. Stefan, S. Jansen, and M. Bollen, “NPP-type ectophosphodiesterases: unity in diversity,” Trends in Biochemical Sciences, vol. 30, no. 10, pp. 542–550, 2005.
[2] M. L. Stracke, A. Arestad, M. Levine, H. C. Krutzsch, and L. A. Liotta, “Autotaxin is an N-linked glycoprotein but the sugar moieties are not needed for its stimulation of cellular motility,” Melanoma Research, vol. 5, no. 4, pp. 203–209, 1995.
[3] S. Jansen, C. Stefan, J. W. M. Creemers et al., “Proteolytic maturation and activation of autotaxin (NPP2), a secreted metastasis-enhancing lysospholipase D1,” Journal of Cell Science, vol. 118, no. 14, pp. 3081–3089, 2005.
[4] L. M. Yuelling and B. Fuss, “Autotaxin (ATX): a multi-functional and multi-modular protein possessing enzymatic lysOPLD activity and matricellular properties,” Biochimica et Biophysica Acta, vol. 1781, no. 9, pp. 525–530, 2008.
[5] K. Nakanaga, K. Hama, and J. Aoki, “Autotaxin-An LPA producing enzyme with diverse functions,” Journal of Biochemistry, vol. 148, no. 1, pp. 13–24, 2010.
[6] L. A. Van Meeteren, P. Ruurs, E. Christodoulou et al., “Inhibition of autotaxin by lysosphophatidic acid and sphingosine 1-phosphate,” Journal of Biological Chemistry, vol. 280, no. 22, pp. 21155–21161, 2005.
[7] T. Sugiuira, S. Nakane, S. Kishimoto, K. Waku, Y. Yoshioka, and A. Tokumura, “Lysosphophatidic acid, a growth factor-like lipid, in the saliva,” Journal of Lipid Research, vol. 43, no. 12, pp. 2049–2055, 2002.
[8] M. Tanaka, Y. Kishi, Y. Takanewaza, Y. Kakubi, J. Aoki, and H. Arai, “Prostatic acid phosphatase degrades lysosphophatidic acid in seminal plasma,” FEBS Letters, vol. 571, no. 1–3, pp. 197–204, 2004.
[9] A. Masuda, K. Nakanuma, K. Izutsu et al., “Serum autotaxin measurement in haematological malignancies: a promising marker for follicular lymphoma,” British Journal of Haematology, vol. 143, no. 1, pp. 60–70, 2008.
[10] K. Nakamura, M. Nangaku, R. Ohkawa et al., “Analysis of serum and urinary lysospholipase D/autotaxin in nphrotic syndrome,” Clinical Chemistry and Laboratory Medicine, vol. 46, no. 1, pp. 150–151, 2008.
[11] K. Nakamura, K. Igarashi, R. Ohkawa et al., “Autotaxin enzyme immunoassay in human cerebrospinal fluid samples,” Clinica Chimica Acta, vol. 405, no. 1–2, pp. 160–162, 2009.
[12] S. Jansen, M. Andries, K. Vekemans, H. Vanbilloen, A. Verbruggen, and M. Bollen, “Rapid clearance of the circulating metastatic factor autotaxin by the scavenger receptors of liver sinusoidal endothelial cells,” Cancer Letters, vol. 284, no. 2, pp. 216–221, 2009.
[13] M. Tanaka, S. Okudaira, Y. Kishi et al., “Autotaxin stabilizes blood vessels and is required for embryonic vasculature by producing lysosphatidic acid,” Journal of Biological Chemistry, vol. 281, no. 35, pp. 25822–25830, 2006.
[14] S. E. Alvarez, S. Milstien, and S. Spiegel, “Autocrine and paracrine roles of sphingosine-1-phosphate,” Trends in Endocrinology and Metabolism, vol. 18, no. 8, pp. 300–307, 2007.
[15] H. Fyrst and J. D. Saba, “An update on sphingosine-1-phosphate and other sphingolipid mediators,” Nature Chemical Biology, vol. 6, no. 7, pp. 489–497, 2010.
[16] T. Clair, H. Y. Lee, L. A. Liotta, and M. L. Stracke, “Autotaxin is an exoenzyme possessing 5'-nucleotide phosphodiesterase/ATP pyrophosphatase and ATPase activities,” Journal of Biological Chemistry, vol. 272, no. 2, pp. 996–1001, 1997.
[17] R. Gijbers, J. Aoki, H. Arai, and M. Bollen, “The hydrolysis of lysospholipids and nucleotides by autotaxin (NPP2) involves a single catalytic site,” FEBS Letters, vol. 538, no. 1–3, pp. 60–64, 2003.
[18] L. A. Van Meeteren and W. H. Moolenaar, “Regulation and biological activities of the autotaxin-LPA axis,” Progress in Lipid Research, vol. 46, no. 2, pp. 145–160, 2007.
[19] J. Aoki, A. Inoue, and S. Okudaira, “Two pathways for lysosphophatidic acid production,” Biochimica et Biophysica Acta, vol. 1781, no. 9, pp. 513–518, 2008.
[20] S. Okudaira, H. Yukiura, and J. Aoki, “Biological roles of lysosphophatidic acid signaling through its production by autotaxin,” Biochimie, vol. 92, no. 6, pp. 698–706, 2010.
[21] K. Hama, K. Bandoh, Y. Kakubi, J. Aoki, and H. Arai, “Lysosphophatidic acid (LPA) receptors are activated differentially by biological fluids: possible role of LPA-binding proteins in activation of LPA receptors,” FEBS Letters, vol. 523, no. 1–3, pp. 187–192, 2002.
[22] J. Lee, I. D. Jung, S. W. Nam et al., “Enzymatic activation of autotaxin by divergent cations without EF-hand loop region involvement,” Biochemical Pharmacology, vol. 62, no. 2, pp. 219–224, 2001.
[23] J. A. Boutin and G. Ferry, “Autotaxin,” Cellular and Molecular Life Sciences, vol. 66, no. 18, pp. 3009–3021, 2009.
[24] S. Jansen, M. Andries, R. Derua, E. Vaelkens, and M. Bollen, “Domain interplay mediated by an essential disulfide linkage is critical for the activity and secretion of the metastasis-promoting enzyme autotaxin,” Journal of Biological Chemistry, vol. 284, no. 21, pp. 14296–14302, 2009.
[25] E. Koh, R. W. Bandle, D. D. Roberts, M. L. Stracke, and T. Clair, “Novel point mutations attenuate autotaxin activity,” Lipids in Health and Disease, vol. 8, article no. 4, 2009.
[26] L. A. Van Meeteren, P. Ruurs, C. Stortelers et al., “Autotaxin, a secreted lysosphopholipase D, is essential for blood vessel formation during development,” Molecular and Cellular Biology, vol. 26, no. 13, pp. 5015–5022, 2006.
[27] H. W. Choi, C. W. Lee, and J. Chun, “Biological roles of lysospholipid receptors revealed by genetic null mice: an update,” Biochimica et Biophysica Acta, vol. 1781, no. 9, pp. 531–539, 2008.
[28] S. Liu, M. Umezuto-Goto, M. Murph et al., “Expression of autotaxin and lysosphophatidic acid receptors increases mammary tumorigenesis, invasion, and metastases,” Cancer Cell, vol. 15, no. 6, pp. 539–550, 2009.
[29] M. L. Stracke, H. C. Krutzsch, E. J. Unsworth et al., “Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulating protein,” Journal of Biological Chemistry, vol. 267, no. 4, pp. 2524–2529, 1992.
[30] M. Umezuto-Goto, Y. Kishi, A. Taira et al., “Autotaxin has lysospholipidase D activity leading to tumor cell growth and motility by lysosphophatidylcholine-induced migration of human breast cancer and melanoma cells,” Molecular Carcinogenesis, vol. 48, no. 9, pp. 801–809, 2009.
[31] M. K. Altman, V. Gopal, W. Jia et al., “Targeting melanoma growth and viability reveals dualistic functionality of the
