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Lysophospholipids (LPLs) are lipid messengers that are present in the extracellular environment and play important roles in cell signaling. By binding to specific receptors, the effects of LPLs are translated into specific cellular responses. Lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) are LPLs that regulate a variety of signaling pathways implicated in both health and disease. As a consequence, LPLs, their receptors and the enzymes that generate or degrade them, are attractive drug targets for various diseases.

This thesis focuses on autotaxin (ATX), the main enzyme responsible for the production of LPA. The studies described in this thesis aim at characterizing the biochemical and functional properties of ATX, to advance our understanding of the molecular actions of ATX in (patho)physiology. This chapter introduces LPA production and signaling, elaborates on ATX processing, activity and regulation, and highlights the role of ATX-LPA receptor signaling in development and disease.
LYSOPHOSPHATIDIC ACID

LPA (monoacyl-sn-glycero-3-phosphate) is a structurally simple phospholipid, composed of a phosphate headgroup, a glycerol backbone and a fatty acyl chain (Fig. 1A). Originally, LPA was known as an intermediate in intracellular lipid metabolism, and only later recognized as an extracellular bioactive lipid mediator and major active component of serum. LPA has growth factor-like activities and stimulates a wide variety of cellular responses; including cell proliferation, survival, migration, morphological changes, wound healing, cytokine and chemokine secretion, increased endothelial permeability and inhibition of gap-junctional communication (1). More than a decade after the initial discovery of LPA as a bioactive lipid mediator, ATX was found to be identical to plasma lysosphospholipase D (lysoPLD), the main enzyme responsible for producing bioactive LPA (2;3).

LPA Production

LPA is present in serum, plasma, saliva, follicular fluid, seminal plasma, mildly oxidized LDL (1) and malignant effusions, such as ascitic fluid from ovarian cancer patients (4;5). LPA is primarily produced in plasma, mainly via lysosphospholipase D (lysoPLD) activity by hydrolyzing lysophosphatidylcholine (LPC) (6). LPC is a lysophospholipid composed of a choline head group, a phosphate group, a glycerol backbone and a fatty acyl chain (Fig. 1B). In the extracellular LPA production pathway, phospholipids are first converted to lysophospholipids by phospholipase A1 (PLA1) or A2 (PLA2) and subsequently these lysophospholipids are hydrolyzed by plasma lysoPLD/ATX to produce LPA (Fig. 1C). Next to this route, LPA can be produced intracellularly as well, via phospholipid hydrolysis by an intracellular PLD and subsequent hydrolysis of the product phosphatidic acid (PA) by PA-selective PLA (Fig. 1D). However, intracellular LPA is thought to be an intermediate for the synthesis of more complex phospholipids and it is unlikely to function as an extracellular mediator (7).

Extracellular Metabolism of LPA

LPA is a mixture of fatty acids that vary in the type of fatty acyl chain (length and saturation) and the linkage to the glycerol backbone (sn-1 or sn-2). LPA species with saturated fatty acids (16:0, 18:0) and unsaturated fatty acids (16:1, 18:1, 18:2, 20:4) have been detected in vivo (8). In serum and plasma, LPA is bound to albumin (9). While human plasma LPA levels are low (10;11), serum LPA levels are significantly higher (9;12). For this reason it has been proposed that LPA in serum is produced by platelets as a result of blood coagulation. It is now clear that increased serum LPA levels upon platelet activation are not due to secretion of LPA from platelet granules. Instead, LPA is generated de novo by sequential actions of phospholipases. First, lysophospholipids (mainly LPC) are produced by activated platelets via PLAs and subsequently these lysophospholipids are hydrolyzed by ATX to produce LPA (13;14). In addition, LPA can be produced by hydrolysis of either PA, released by platelets, or LPC that is present on erythrocyte membranes. However, these two pathways make minor contributions to LPA production in blood (13).

Steady-state plasma LPC levels (>100 mM) are about 1000-fold higher than the levels of LPA, while ATX is constitutively active (12-14). This suggests a tightly controlled equilibrium between production, degradation and clearance. Indeed, LPA is rapidly degraded by membrane-bound phosphatases, mainly lipid phosphate phosphohydrolase type 1 (15), and ATX is cleared from the circulation within minutes (16). Moreover, the low plasma LPA levels
Figure 1: Structure and production of LPA. (A) Chemical structure of 1-acyl-2-hydroxy-sn-glycero-3-phosphate (LPA). In this example 1-oleoyl LPA (LPA 18:1) is shown. Note the presence of the fatty acyl chain on the sn-1 position of the glycerol backbone, which is the most common form. (B) Chemical structure of 1-oleoyl-2-hydroxy-sn-glycero-3-phosphocholine (LPC). In this example 1-oleoyl LPC (LPC 18:1) is shown. The red arrow depicts where LPC will be hydrolyzed by ATX. (C), (D) LPA is produced via two major pathways: an extracellular (C) and intracellular (D) route. Several LPA species can be produced via these pathways, depending on the length and saturation level of the fatty acyl chain of the phospholipids. **Note that phospholipids contain two fatty acyl chains, while lysophospholipids only have a single fatty acyl chain. Examples of phospholipids are phosphatidycholine, phosphatidylethanolamine and phosphatidylserine, which all have different head groups connected to the phosphate. This figure is adapted from (7).**
suggest high capacity of peripheral tissues to take up or metabolize circulating LPA (17). Together, these processes will contribute to the maintenance of low levels of circulating LPA.

**LPA Receptors**

LPA evokes a wide variety of cellular responses through binding to specific type I, rhodopsin-like G protein-coupled receptors (GPCRs), which are seven-transmembrane cell-surface receptors that signal via heterotrimeric G proteins. Subsequently, these G proteins can activate multiple signaling cascades (18). Until now, six LPA receptors have been identified and termed LPA$_{1-6}$, which can be subdivided into two subfamilies (Fig. 2A). The presence of two LPA receptor subfamilies suggests that LPA receptors have evolved from distinct ancestor genes. LPA$_{1-3}$ belong to the “endothelial differentiation gene” (EDG) family and show about 50% amino acid homology. They are the “classical” LPA receptors and were formerly called EDG-2, EDG-4, EDG-7 respectively. The five additional members of the EDG-receptor subfamily are receptors specific for the bioactive lysophospholipid S1P, a small lipid similar to LPA but with a sphingosine instead of a glycerol backbone. The newly identified LPA receptors LPA$_{4-6}$ (or P2Y9, GPR92, P2YS, respectively) are more closely related to the purinergic (P2Y) receptors, sharing about 35% amino acid identity. They were previously thought to bind nucleotides like their purinergic receptor family members (19;20).

**Biological Effects of LPA**

LPA signaling through GPCRs activates a wide variety of signaling pathways, ranging from cell proliferation and migration to neurite remodeling and cytokine production (21;22). The effects include developmental and (patho) physiological processes and they involve basic cellular responses as well as multiple organ systems. LPA receptors show both overlapping and distinct signaling properties and tissue expression, with an outcome of LPA signaling depending on the LPA receptor expression profile in the cell (19;20). Some of the major biological effects of LPA are summarized in Table 1.

The main LPA-induced signaling pathways and there downstream effects are shown in Fig. 2B. They include: (i) cell proliferation via the $G_i$-linked Ras-MAPK (mitogen-activated protein kinase) pathway (23;24); (ii) cell survival via $G_i$-mediated activation of both the PI3K (phosphatidylinositol 3-kinase) - Akt/PKB (protein kinase B) pathway and the MAPK pathway (25;26); (iii) cytoskeletal remodeling and cell migration via the $G_{12/13}$-RhoA pathway (27;28) and $G_q$-mediated Rac activation (29); (iii) $G_{q}$-mediated hydrolysis of PIP$_2$ (phosphatidylinositol-bisphosphate) by PLC (phospholipase C), with consequent calcium mobilization and PKC (protein kinase C) activation (30;31). Furthermore, LPA stimulation can induce changes in cAMP (cyclic adenosine monophosphate) levels via $G_i$ or $G_{q}$ subunits and production of growth factors and cytokines (reviewed in (19)).

**AUTOTAXIN**

ATX was identified as an autocrine motility factor from A2058 melanoma cell-conditioned medium about two decades ago (32). Its mode of action was a mystery at the time and it took another decade until it was discovered that ATX was identical to plasma lysophospholipase D$_2$, responsible for the production of LPA (2;3). ATX, or NPP2, is a member of the ecto-nucleotide pyrophosphatase/phosphodiesterase ((E)NPP) family, consisting of seven structurally related ecto-enzymes. NPPs are usually present at the cell surface as type-I or type-II trans-
membrane proteins and are known to hydrolyze pyrophosphate or phosphodiester bonds in nucleotides and their derivatives. Despite their structurally related catalytic domain, each NPP enzyme has well-defined substrate specificity. Currently, it is widely accepted that ATX, although capable of hydrolyzing nucleotides in vitro, primarily functions as a lysoPLD in vivo and thus represents a unique family member. The closest relatives of ATX, NPP1 and

Figure 2: LPA receptor signaling. (A) Phylogenetic tree of human LPA receptors with indicated amino acid identity to LPA1. The EDG family receptors are depicted in light grey and the purinergic receptors in dark grey. This figure is adapted from (19). (B) Major signaling pathways of the LPA receptors, showing the distinct G proteins and their corresponding downstream effectors. G proteins are heterotrimeric proteins composed of 3 subunits: α, β and γ. RhoGEF: Rho-specific guanine nucleotide exchange factor. This figure is adapted from (19).
NPP3, convert adenosine triphosphate (ATP) into pyrophosphate (PP\(_i\)) and thereby regulate mineralization and calcification in bone. NPP6, as well as NPP7, is a choline-specific ectophospholipase C, probably serving catabolic functions. Activities of NPP4 and NPP5 are currently not defined (33).

**ATX Processing**

ATX is synthesized as a pre-pro-enzyme; the N-terminal signal peptide is removed by a signal peptidase, the protein is further trimmed by a furin-type protease, traffics along the classical export pathway and is subsequently secreted as an extracellular enzyme into the circulation (34-36). ATX is a heavily glycosylated protein and glycosylation of asparagine-residue 524 (Asn524) is essential for catalytic activity (37). ATX has a rapid turnover; it is cleared from the circulation within minutes and subsequently degraded in the liver. The constant levels of ATX and LPA in serum suggest a continuous synthesis of ATX (16). The cellular source of plasma ATX remains to be identified, but it is likely to originate from the abundant ATX-expressing lymphatic high endothelial venules (38) and/or adipose tissue (39). Indeed, adipose-ATX contributes significantly (though not completely) to circulating LPA levels, as demonstrated in adipose-specific ATX knockout mice that show around 40% reduction in plasma LPA levels (40).

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<td>Transformation (81)</td>
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<td>Wound healing (87)</td>
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<td>Ion channel activity (91)</td>
<td>Nerve demyelination and initiation of pain (74)</td>
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<td><strong>Vascular system</strong></td>
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<td>Regulation of food intake (67)</td>
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Table 1: Major biological LPA signaling-mediated functions. Note that this list focuses on the main effects of LPA and is not complete. However, the remarkable diversity of effects points to the multifunctional signaling function of LPA. This table was adapted and adjusted from (19;21).
ATX Expression

Expression of ATX is most abundant in brain, placenta, ovary and small intestine; while intermediate expression levels are detected in kidney, prostate, testis, pancreas, colon and lung (41). During mouse development, ATX is first detectable at embryonic day 8.5 (E8.5) in the extra-embryonic yolk sac and at E9.5 in the floor plate of the neural tube (42). At later embryonic stages and in adulthood, high ATX expression is observed in choroid plexus epithelium (35;42-44). ATX has been detected in biological fluids, like cerebrospinal fluid (35;45), seminal fluid (46) and ascites (47).

Phosphodiesterase and LysoPLD Activity

ATX has both phosphodiesterase (PDE) and lysoPLD activity in vitro; it hydrolyzes nucleotide and lysophospholipid substrates, respectively, with threonine-residue 210 (Thr210) as the catalytic center for both activities. The PDE activity of ATX, however, does not seem to be of biological importance, as the apparent affinity for LPC is much higher than that for nucleotides (48). ATX has also been shown to hydrolyze sphingosylphosphorylcholine (SPC) and produce S1P in vitro (49). However, physiological significance is ambiguous due to a thousand-fold higher K_m of ATX for SPC than the normal SPC levels in plasma and serum (50). Thus, the biological roles of ATX are thought to be fully attributable to LPA production and subsequent signaling through LPA receptors.

ATX Crystal Structure

The recently obtained crystal structures of rat and mouse ATX identify a compact four domain-based architecture of two somatomedin B-like (SMB) domains, a central catalytic PDE domain and a nuclease-like (NUC) domain (Fig. 3A) (51;52). The PDE domain extensively interacts with the SMB-1 and SMB-2 domains on one side and with the NUC domain on the other side. A short loop is connecting the SMB and PDE domains and a long “lasso loop”, wrapped around the NUC domain, connects the PDE and NUC domains. The Asn524-linked glycan chain further reinforces the interdomain interactions, in agreement with the finding that this glycan is essential for catalytic activity (37).

The catalytic domain of ATX is similar to that of the bacterial Xanthomonas axonopodis NPP, a structural and evolutionary relative to alkaline phosphatase (AP). The crystal structure of ATX reveals the presence of two catalytic zinc ions coordinated by conserved residues, in close proximity to Thr210. As in X. axonopodis NPP and many other enzymes, the ions in this conserved bimetallo site participate in bond-breaking steps (51;53).

The catalytically inactive NUC domain has been thought to be involved in substrate binding and presentation (54). It is now clear that the rigid PDE-NUC domain interface merely functions to stabilize the thermodynamically unstable structure of the hydrophobic pocket of ATX, without directly contributing to the active site formation. Moreover, this stabilizing effect could be the reason why the hydrophobic lipid-binding pocket of ATX is always accessible, while most lipases have a closed pocket that only opens upon substrate binding (51;52).

Substrate Specificity

ATX hydrolyzes both nucleotide and phospholipid substrates in vitro (48). Substrate discrimination of ATX and the diverse substrate preferences of NPP family members have been a long time mystery. The ATX crystal structure reveals that both nucleotides and lipids partially
Figure 3: The structure of ATX. (A) The upper panel shows the crystal structure of ATX bound to LPC 14:0 with the N- and C-terminus, the zinc ions (yellow spheres) in the active site, the calcium ion (blue sphere) bound to the EF hand-like motif indicated. LPC, the essential glycan chain of Asn524, the integrin-interaction motifs LDV and His119 (close to the RGD motif) are depicted in a stick-and-sphere representation. The lower panel shows a schematic overview of the domain organization, with the active site residue Thr210, the 18-residue deletion (specific for ATX in the NPP family and responsible for formation of the lipid binding pocket) and Asn524 (containing the glycan chain essential for interdomain interactions between PDE and NUC). This figure was prepared using Pymol (www.pymol.org), adapted from (58). (B) Surface representation of ATX bound to LPC 14:0, with the acyl chain pointing into the viewing plane and the tunnel pointing upwards (left panel). LPC is shown in a sphere model. Domain colors are the same as in Fig. 3A. The right panel shows a zoom-in on the ATX active site showing LPA in the tunnel (again pointing upwards), which forms a T-junction with the substrate binding pocket containing LPC (with the phosphate group bound to the active site in the lower right corner and the acyl chain pointing inwards). The roof of the tunnel is partially made by residues of the SMB-1 domain, depicted in purple. This figure was prepared using Pymol (www.pymol.org), adapted from (58).
share the same binding pocket. The acyl chain of lipid substrates form additional hydrophobic contacts with ATX, suggesting that the product LPA has a higher affinity for ATX than nucleotide substrates and can only be displaced by lysophospholipid substrates like LPC. This would explain why LPA acts as an inhibitor of ATX activity against various artificial substrates (36) but not of LPC hydrolysis (55), determining LPA as a “substrate-specifying factor” (51).

The presence of an 18 amino acid insertion loop in all of the NPP family members, except ATX, is a major determinant for substrate specificity among the family. This insertion loop blocks the entry of the hydrophobic binding pocket, suggesting that the hydrophobic pocket is an extension of the nucleotide binding site. The absence of the loop in ATX enables the enzyme to accept both nucleotides and lysophospholipids as a substrate and to use the same catalytic site for both activities. Moreover, this pocket can only accommodate mono-acyl but not diacyl phospholipids, providing substrate specificity for phospholipids with a single acyl chain and with an optimal length of 14 carbons (51;52).

Close to the hydrophobic pocket of ATX is a narrow tunnel, which is formed by the PDE and SMB-1 domains (Fig. 3B). An SMB-deletion mutant of ATX is ten-fold less sensitive to LPA inhibition, suggesting a role for the SMB domain and the tunnel in substrate recognition or product release (51). Indeed, Nishimasu and colleagues imply the presence of LPA in this hydrophobic tunnel, which suggests that the tunnel could function as a product exit route. Again, due to the presence of the insertion loop, the other NPP family members lack this tunnel. Another option would be that the tunnel serves as an entrance site for LPC substrates released from cells, which could function as a shuttle mechanism for substrate entrance versus product release to deliver LPA directly to LPA receptors on the cell membrane (52).

**ATX Interactions**

ATX has a flat molecular surface on the side of the tunnel, which is suggested to function as a cell membrane-binding platform to provide LPA directly to its receptors (52). Next to this model of direct binding, ATX has been suggested to bind to cells via interaction with integrins. T cells are able to interact with ATX in an integrin \(\alpha_4\beta_1\)-dependent manner, suggesting a model in which secretion of ATX and subsequent localized LPA production promotes lymphocyte motility. This interaction is thought to involve a Leu-Asp-Val (LDV) integrin-binding motif on the surface of the PDE domain of ATX (38). In addition to lymphocytes, ATX can bind to activated platelets in an integrin \(\beta_3\)-dependent manner, which could be mediated by an Arg-Gly-Asp (RGD) sequence motif present in ATX (56).

The ATX crystal structure revealed that the SMB domains are the primary mediators of the \(\beta_3\)-mediated ATX-integrin interaction. However, the binding mode is structurally distinct from the classical RGD-mediated integrin binding and involves a wider portion of the surface of the SMB-2 domain (51). Recently, platelets have been shown to bind ATX in an integrin-dependent manner with subsequent increase in local LPA production. Moreover, it is suggested that this ATX-integrin interaction even enhances LPA production, possibly by reorientation of the SMB domains to speed up catalysis or product release (57). Next to interactions with integrins, ATX is able to bind heparin. In chapter 5 we discuss our latest insight in ATX-heparin interactions and propose a third mechanism of localized LPA signaling by sequestering ATX to target cells via heparin sulphate proteoglycans. Together, these
models of ATX recruitment to the cell surface suggest that ATX not only drives the production of LPA, but also guides specificity of LPA signaling. Alternatively, the function of these interactions could be non-catalytic signaling of ATX via integrins (58).

**ATX Isoforms**
The human gene encoding ATX, *ENPP2*, is located on chromosome 8 and is organized in 27 exons. Alternative splicing of the *ENPP2* gene gives rise to three isoforms; ATXα, ATXβ and ATXγ. The originally isolated ATX isoform termed “autocrine motility factor”, cloned from A2058 melanoma cells, was called ATX-melanoma or ATXα (32;59). Later, ATX-teratocarcinoma (ATXβ) was cloned from a teratocarcinoma cell line (41). This isoform is identical to plasma lysoPLD and currently the most widely studied isoform. A third, brain-specific isoform PD-Iα was identified and termed ATXγ (44;60). The ATXα and ATXγ isoform are characterized by the presence of exon 12 and 21, respectively. ATXα represents the longest isoform with a 52 amino acid insert in the PDE domain. ATXγ has an insert of 25 amino acids in the NUC domain. ATXβ was thought to be the shortest isoform, lacking both exon 12 and 21. Recently, two new isoforms have been identified: ATXδ and ATXε. ATXδ has a deletion of 4 amino acids in the lasso loop and ATXε is characterized by the 52 amino acid insert encoded by exon 12 together with the 4 amino acid deletion. Of all isoforms, ATXα represents the longest isoform and ATXδ the shortest (Fig. 4). ATXβ and ATXδ seem to be vastly expressed, ATXγ moderately, while ATXα and ATXε are much less abundant (61). The ATXα isoform is of special interest, since it contains a large insert in the heart of the catalytic domain. The unique biochemical properties of the ATXα isoform will be discussed in chapter 5.

**ATX-LPA RECEPTOR SIGNALING IN DEVELOPMENT**

**ATX Knockout Mice**
Gene targeting studies in mice show a crucial role for ATX in embryogenesis. *Enpp2* deficiency causes embryonic lethality at E9.5 due to vascular defects in both yolk sac and embryo. The vascular network normally present in the yolk sac is completely absent and replaced by blood patches, blood vessels in the embryo are enlarged and the neural tube is malformed.

![Figure 4: ATX isoforms](image-url)
Moreover, the heterozygous mice show half-normal plasma LPA levels, indicating that ATX is the main enzyme producing LPA in the circulation (62-64). Transgenic mice expressing the inactive mutant ATX-T210A display the same lethal phenotype, confirming that catalytic activity of ATX is crucial for vascular development (65).

**LPA Receptor Knockout Mice**

Loss of LPA\(_1\) in mice results in 50% neonatal lethality and a reduced body size of surviving pups (66), which was attributed to defective suckling but has now been shown to be caused by a reduced food intake as well (67). In addition, LPA\(_3\) has been implicated in neurogenesis, as a specific variant of the Lpa\(_3\) KO strain shows defects in cortical development (68). Lpa\(_2\) knockout mice are viable and without any phenotypic abnormalities, while Lpa\(_1\)/Lpa\(_3\) double knockout mice do not show additional phenotypic abnormalities next to the defects found in the Lpa\(_2\) single knockout mice. Thus, LPA\(_2\) is not essential for mouse development, but rather acts redundantly with LPA\(_1\) (69).

LPA\(_3\) signaling, though, is crucial for embryo implantation and spacing. While Lpa\(_3\) knockout mice appear normal, the Lpa\(_2\)-deficient females show a reduced litter size due to delayed embryo implantation and embryo crowding (70). Lpa\(_1\)/Lpa\(_3\)/Lpa\(_3\) triple knockout mice show defects in male reproductive function, next to the phenotypes consistent with single receptor deletants (71). Lpa\(_3\)-deficient mice display abnormalities in the blood and lymphatic vascular system, causing partial lethality of embryos and newborn pups (72). The latter phenotype shows some similarities to, but is less severe than the Enpp2 knockout mice.

**ATX-LPA Receptor Signaling in Vascular Development**

The vascular defects observed in Enpp2-deficient embryos have also been found in G\(_{13}\) knockout mice (73). Absence of G\(_{13}\) does not influence the formation of endothelial cells, but affects the growth and sprouting of endothelial cells to form an organized vascular network (73). While initial blood vessel formation is not affected in the Enpp2 deficient mice, newly formed blood vessels fail to mature in the absence of ATX (62;63). This suggests that ATX and LPA play a role in angiogenesis. However, ATX and LPA are suggested to function in stabilizing preformed blood vessels, rather than being angiogenic factors (63). Thus, loss of LPA-G\(_{13}\) signaling is likely to underlie the vascular defects of Enpp2 knockout embryos, in particular defects in the formation and stabilization of blood vessels.

**ATX-LPA RECEPTOR SIGNALING IN (PATHO)PHYSIOLOGY**

ATX-LPA signaling has been implicated in a wide range of physiological and pathological processes, ranging from vascular and neural development (62-64), lymphocyte trafficking (38), neuropathic pain (74), fibrosis (75), hydrocephalus (76) and cholestatic pruritus (77) to tumor initiation and progression (78-81). ATX may function as a potential diagnostic marker since the serum or plasma levels are increased in some physiological and pathological conditions like chronic liver disease (82), obesity (39;40), cancer (83) and pregnancy (84). In addition, the implication of the ATX-LPA axis in diverse pathophysiological processes has put forward the identification of ATX as a valuable therapeutic target. The crystal structure has revealed a wealth of important information regarding substrate binding and selectivity, which will boost the design and optimization of effective ATX inhibitors in the near future.
A prominent area of research interest is the emerging role of ATX-LPA receptor signaling in cancer. With its growth-factor like activities, LPA stimulates multiple signaling pathways that could contribute to tumor initiation and progression as well as metastasis. Indeed, LPA receptor expression and signaling, aberrant LPA production and ATX expression have all been linked to cancer (1). As discussed above, most evidence is pointing to a role of the ATX-LPA signaling axis in vivo in the vascular system. This raises the question if ATX could contribute to the progression of tumors by stabilizing blood vessels in tumors or the tumor microenvironment, in addition to its effect on proliferation and migration. Additional studies such as deletion of non-EDG LPA receptors (LPA\textsubscript{4-6}) and their combinations, as well as tissue specific or inducible deletion of both ATX and/or LPA receptors, are awaited to sort out the mechanism of the ATX-LPA signaling axis and its role in development and pathology. Details about the role of ATX-LPA receptor signaling in cancer will be further discussed in chapter 2.

**THESIS OUTLINE**

The ATX-LPA receptor axis has a wide implication in health and disease. The studies described in this thesis focus on the biochemical and functional properties of ATX to increase our understanding of its molecular actions. **Chapter 2** reviews the latest insights of ATX and LPA receptor signaling in cancer. The prognostic value of ATX protein expression in breast cancer is explored in **chapter 3. Chapter 4** describes the development and optimization of a first-generation ATX activity-based probe for in vivo screening of ATX activity. Next, the specific characteristics of the ATX\textalpha isoform have been studied: intradomain cleavage of ATX\textalpha and high-affinity binding to heparin are discussed in **chapter 5**, and the SH3 domain-mediated protein-interaction capacity of ATX\textalpha is described in the **chapter 6**. In **chapter 7**, all results presented in this thesis are summarized and discussed.
REFERENCES

molecular recognition.


