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LIPID GROWTH FACTOR SIGNALING

Our group has a longstanding interest in the lipid growth factor lysophosphatidic acid (LPA), its signaling properties and role in health and disease. LPA signals through at least six distinct G protein-coupled receptors and thereby stimulates the proliferation, migration and survival of many cell types, both normal and malignant. LPA is produced extracellularly from lysophosphatidylcholine by a secreted lysophospholipase D (lysoPLD), named autotaxin (ATX), which was originally identified as an autocrine motility factor for melanoma cells. The ATX-LPA signaling axis is essential for vascular development and is a significant effector of tumor progression in mice. As such, ATX qualifies as an attractive target for therapy. Our current research focuses on the regulation of ATX and its *in vivo* functions as well as the characterization of novel LPA receptors and downstream effectors. Our longstanding collaboration with the structural biology group of A. Perrakis (Division of Biochemistry) has recently led to the elucidation of the crystal structure of ATX, an important breakthrough that provides new insights into ATX structure-activity relationships. Our studies should lead to novel ways of interfering with ATX-LPA receptor signalling and with inappropriate LPA production in the tumor-stroma microenvironment.

Autotaxin, a secreted lysophospholipase D implicated in tumor progression

We previously have established that ATX is essential for vascular development in mice. Increasing evidence points to a significant role of the ATX-LPA receptor axis in cancer. In particular, enforced overexpression of ATX or individual LPA receptors promotes tumor progression in experimental animals, while elevated expression of certain LPA receptors is associated with poor clinical outcome in breast cancer patients. Obviously, there is now an urgent need for pharmacological inhibitors of ATX and, furthermore, a better understanding of how ATX activity is regulated and how newly produced LPA is presented to its cognate receptors. We therefore set out to discover potent inhibitors and to determine the crystal structure of ATX.

ATX inhibitors

To identify small-molecule inhibitors of ATX, we have performed high-throughput screening of chemical compound libraries (collaboration Huib Ovaa). Among the positive hits were several new chemical structures that have been optimized to reach IC₅₀ values in the nanomolar range. Specificity, selectivity and toxicity of these pharmacological inhibitors are being further evaluated in cell-based and animal studies. A boronic-based ATX inhibitor was found to rapidly lower plasma LPA levels in mice, indicating that LPA turnover in the circulation is much more dynamic than previously appreciated.

Crystal structure of ATX The LPA-generating activity of ATX has been well characterized, but the molecular basis of substrate recognition and catalysis, and how ATX may interact with target cells has been elusive. Through collaborations with the groups of Anastassis Perrakis (Division of Biochemistry), Andrew Morris (University of Kentucky, USA) and Huib Ovaa (Division of Cell Biology II), the crystal structure of ATX has been determined, alone and in complex with a small-molecule inhibitor. The ATX structure reveals a unique hydrophobic lipid-binding pocket and allowed us to map key residues required for catalysis and selection between nucleotide and phospholipid substrates. ATX was found to interact with cell-surface integrins via its N-terminal somatomedin-B-like domains, using an atypical mechanism. Our results define determinants of substrate discrimination by ATX and its family members, suggest how ATX promotes localized LPA signaling, and enable new approaches to target ATX by small-molecule inhibitors.

The melanoma-derived ATX isoform The prototypic and best studied isoform of ATX is identical to plasma lysophospholipase D. The original “melanoma-derived” isoform of ATX (ATX_m) contains a 52-residue polybasic insert in the catalytic domain. The function of this polybasic stretch remains enigmatic to date. We find that the ATX_m insert is cleaved by the protease furin, yet cleavage does not affect the catalytic activity of ATX_m. Proteolytic cleavage of ATX_m likely serves to modulate

the interaction of ATXm with negatively charged heparan sulphate proteoglycans at the cell surface. We are currently exploring this scenario.

LPA receptor signaling

LPA-RhoA signaling: CLIC4 as a new player

LPA receptors couple to the G₁₃-RhoA pathway to regulate the actin cytoskeleton. We previously reported that LPA-induced RhoA activation is consistently accompanied by rapid recruitment of “Chloride Intracellular Channel” protein 4 (CLIC4) to the plasma membrane. CLIC4 is a soluble protein structurally related to omega-type glutathione-S-transferases (GSTs) and implicated in various biological processes, ranging from chloride channel formation to epithelial and endothelial morphogenesis. We found that Rho-dependent CLIC4 translocation depends on conserved residues, whose equivalents are critical for the enzymatic function of GSTs. This suggests that CLIC4 may function as a RhoA-regulated transferase that catalyses a yet unknown chemical reaction. Through yeast two-hybrid screening and pull-down assays, we have identified and validated novel binding partners of CLIC4 that shed new light on its possible function(s) in receptor signalling and morphogenesis.

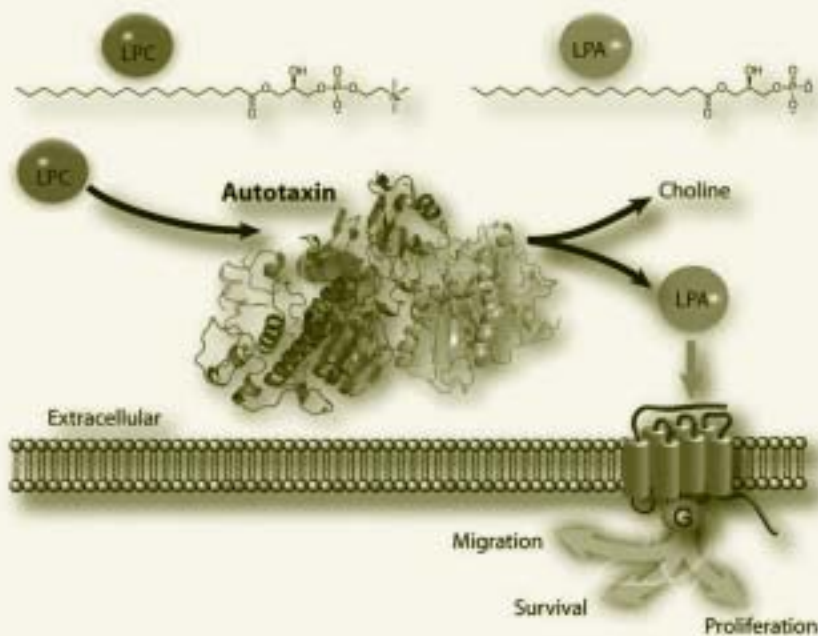


Figure 2: The ATX-LPA receptor signaling system. The lipid mediator LPA arises from the cleavage of lysophosphatidylcholine (LPC) by autotaxin (ATX). LPA acts on specific G protein-coupled receptors to stimulate cell proliferation, migration and survival.

Novel LPA receptors: LPA5 and P2Y5

LPA is a potent chemoattractant for many cell types. In contrast, the novel LPA₅ receptor was found to mediate inhibition of melanoma cell chemotaxis. LPA₅ stimulation leads to a persistent rise in cAMP, which can account at least in part for the inhibitory action of this receptor. Thus, selective agonists of LPA₅ may prove useful to counteract the migration of tumor cells in which LPA₅ is the predominant receptor.

Another novel LPA receptor, termed LPA₆ (encoded by *P2RY5*), couples specifically to RhoA activation and has been implicated in tumor suppression. We are in the process of generating conditional *P2ry5* knockout mice to establish the importance of this unique LPA receptor in tumor development (collaboration P. Krimpenfort, Division of Molecular Genetics).

Publications

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