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## Comment

# Lysophospholipids in the limelight: autotaxin takes center stage

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**Lysophosphatidic acid (LPA) is a serum phospholipid that evokes growth factor–like responses in many cell types through the activation of its G protein–coupled receptors. Although much is known about LPA signaling, it has remained unclear where and how bioactive LPA is produced. Umezu-Goto et al. (2002, this issue, page 227) have purified a serum lysophospholipase D that generates LPA from lysophosphatidylcholine and found it to be identical to autotaxin, a cell motility–stimulating ectophosphodiesterase implicated in tumor progression. This result is surprising, as there was previously no indication that autotaxin could act as a phospholipase.**

Lysophospholipids have often been viewed with suspicion since their accumulation in the plasma membrane leads to necrosis. One notable exception, however, is lysophosphatidic acid (LPA;* monoacyl-glycerol-3-phosphate), which has emerged as an extracellular signaling lipid that evokes hormone- and growth factor–like responses in almost every mammalian cell type (Moolenaar, 1999). It does so by activation of its cognate G protein–coupled receptors (GPCRs), termed LPA$_1$/Edg2, LPA$_2$/Edg4 and LPA$_3$/Edg7, with LPA$_1$/Edg2 being the first identified and most widely expressed subtype (Contos et al., 2000). The cellular responses to LPA are remarkably diverse, ranging from cell proliferation and survival to induction of neurite retraction and inhibition of gap junctional communication (Moolenaar, 1999; Contos et al., 2000). Although most often associated with proliferative responses, LPA also stimulates cell motility and migration. Cell migration is driven by signaling pathways controlled by Rho GTPases (Nobes and Hall, 1999) and is fundamental to many normal and pathophysiological processes. Thus, migration plays a central role not only in embryonic development but also in the progression of tumors from a noninvasive

to an invasive and metastatic phenotype. The role of LPA as a "motility factor" is highlighted by its ability to stimulate Rho/Rac-dependent invasion of tumor cells across a monolayer of normal cells and to promote wound healing both in vitro and in vivo (Stam et al., 1998; Sturm et al., 1999).

Although much progress has been made in understanding LPA signaling, the mechanism of LPA production/secretion has remained unsolved. It has long been known that LPA is a major constituent of serum and originates from platelet activation. LPA also accumulates in conditioned media from cultured cells, whereas its levels are elevated in ascitic fluids from cancer patients, suggesting a role for LPA in the spread of interperitoneal malignancies (for references see Moolenaar, 1999). More than 15 years ago, Tokumura and coworkers discovered a lysophospholipase D (lysoPLD) activity in plasma that generates LPA from lysophosphatidylcholine (LPC; Tokumura et al., 1999). A bacterial secreted PLD can generate LPA from preexisting LPC in the outer membrane leaflet of its target cells (van Dijk et al.,1998), but unlike plasma lysoPLD, bacterial PLD also attacks diacylphospholipids. Plasma lysoPLD plays a key role in the production of LPA following platelet activation (Sano et al., 2002), but its molecular identity has never been determined.

A major step forward has now been made by Aoki and coworkers (Umezu-Goto et al., 2002) who report the purification of lysoPLD from bovine serum. Surprisingly, lysoPLD appears to be identical to the "autocrine motility factor" autotaxin (ATX), a widely expressed ectophoshodiesterase that was previously thought to act through nucleotide signaling (see below). The significance of this finding should not be underplayed as it not only reveals a novel function for ATX but also interlinks previously unconnected areas of cell biology. ATX was originally isolated as a 125-kD glycoprotein that stimulates tumor cell motility in a pertussis toxin–sensitive manner (for review see Stracke et al., 1997), whereas more recent evidence supports an in vivo role for ATX in tumor progression and metastasis (Nam et al., 2000). In addition, ATX promotes angiogenesis in vivo and may contribute to tumor growth by promoting new blood vessel formation (Nam et al., 2001). Interestingly, expression analysis has suggested a role for ATX in oligodendrocyte differentiation and myelination (Fuss et al., 1997), a function also ascribed to the prototypic LPA receptor LPA$_1$/Edg2 (Contos et al., 2000).

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*Abbreviations used in this paper: ATX, autotaxin; GPCR, G protein–coupled receptor; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; lysoPLD, lysophospholipase D; NPP, nucleotide pyrophosphatase and phosphodiesterase; PC, phosphatidylcholine; PLD, phospholipase D.

Table I. **Autotaxin/lysoPLD and family members**

| Enzyme | Other names | Proposed biological roles | Expression |
|---|---|---|---|
| ATX/lysoPLD | NPP2, PD-1α | Tumor cell motility, metastasis[a], angiogenesis[b], myelination[c] | Nearly ubiquitous (not in liver and thymus); up-regulated by BMP-2, bFGF[b], retinoic acid[d], and Wnt-1 signaling[d]; down-regulated by interferon-γ |
| PC-1 | NPP1 | Bone calcification | Nearly ubiquitous (not in lung and ovary) |
| gp130[RB13-6] | NPP3, PD-1β, B10 | Glial differentiation, cell motility/invasion[e] | Nearly ubiquitous (not in heart, lung and ovary) |

For review and primary references see Stracke et al. (1997), Goding et al. (1998), and Bollen et al. (2000). Two putative new members, NPP4 and NPP5 (Bollen et al. 2000), are not included.
[a]Nam et al. (2000).
[b]Nam et al. (2001).
[c]Fuss et al. (1997).
[d]Tice et al. (2002).
[e]Deissler et al. (1999).

ATX/lysoPLD belongs to the nucleotide pyrophosphatase and phosphodiesterase (NPP) family of ectoenzymes, which to date consists of three members (Goding et al., 1998; Bollen et al., 2000; Table I). These enzymes hydrolyze phosphodiester and pyrophosphate bonds, typically found in ATP and ADP, and thereby generate nucleoside 5′-monophosphates. The NPPs are transmembrane proteins with a very short $NH_2$-terminal region, a single transmembrane domain, two cysteine-rich somatomedin B–like domains possibly involved in dimerization, and a large catalytic domain. Soluble ATX/lysoPLD is derived from the membrane-bound form by extracellular proteolytic cleavage (Stracke et al., 1997; Fig. 1).

Until now, there were no indications that ATX could function as a lysoPLD, since its sequence lacks the conserved motifs that characterize the PLD superfamily. Instead, ATX was thought to act by influencing extracellular nucleotide metabolism and/or recycling. With hindsight, however, this idea was perhaps not too appealing. Although ATP, ADP, and adenosine are bona fide GPCR ligands, it is difficult to envision how altered nucleotide processing in the extracellular environment could account for the observed biological effects of ATX. The report by Umezu-Goto et al. (2002) now provides conclusive evidence that the biological effects of recombinant ATX/lysoPLD are mediated by newly generated LPA, negating the need to invoke a role for extracellular nucleotide metabolism.

The major physiological substrate for ATX/lysoPLD, LPC, is secreted by the liver and circulates at high concentrations in plasma, complexed to albumin and lipoproteins. Umezu-Goto et al. (2002) detect significant amounts of LPC and phosphatidylcholine (PC) in serum-free superna-

Figure 1. **Production of bioactive LPA by autotaxin/lysoPLD.** ATX/lysoPLD is a type-II transmembrane glycoprotein that is proteolytically cleaved, as indicated, to yield a soluble exoenzyme. Soluble ATX/lysoPLD hydrolyzes carrier-bound and membrane-associated LPC (and other lysophospholipids) to generate LPA. Newly produced LPA acts on its own G protein–coupled receptors and thereby evokes numerous cellular responses, including Ras-mediated cell proliferation and Rho/Rac-regulated cell migration. Excess LPA is converted into monoacylglycerol (MAG) by membrane-bound lipid phosphatases. See text for further details.
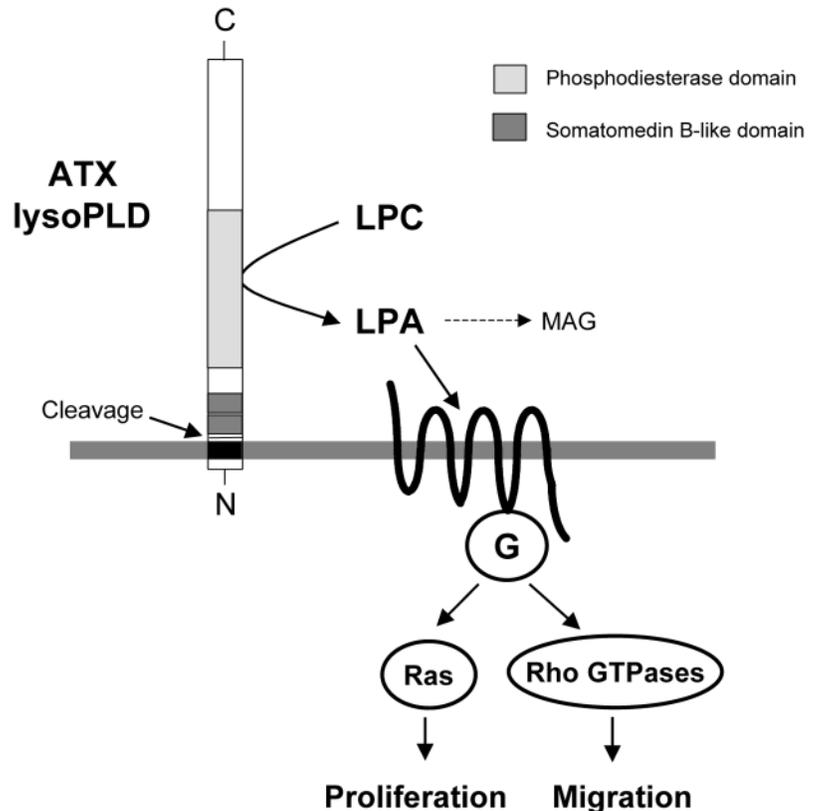
tants from tumor cells. The source of this "secreted" LPC and PC is unknown, although it seems likely that both lipids are constituents of shed microvesicles. This suggests that ATX/lysoPLD hydrolyzes not only carrier-bound and free LPC, but also membrane-associated LPC.

The connection that has now been established between a NPP family member and the production of a bioactive lysophospholipid raises new questions that call for further study. A compelling question is whether the other NPP family members, PC-1/NPP1 and gp130[RB13–6]/NPP3, have a similar phospholipase function. PC-1/NPP1 plays a role in bone calcification (Goding et al., 1998), whereas gp130[RB13–6]/NPP3 has been implicated in glial differentiation (Deissler et al. 1999; Table I). Although it is not immediately clear how ectophospholipase activity could account for these biological effects, the report by Umezu-Goto et al. (2002) paves the way toward testing this notion. Other open questions concern the regulation of ATX/lysoPLD activity and expression. Is the membrane-bound form ATX/lysoPLD catalytically active and how is its cleavage regulated? If the soluble form of ATX/lysoPLD is constitutively active, the question arises as to how extracellular LPA accumulation is kept in check. One scenario is that phospholipid ectophosphatases (Waggoner et al., 1999) cooperate with ATX/lysoPLD to control the local concentration of receptor-active LPA. Finally, it is noteworthy that ATX/lysoPLD mRNA is up-regulated by peptide growth factors, retinoic acid, and Wnt-1 signaling (at least in a mammary epithelial cell line; Tice et al., 2002; Table I). This suggests unexplored connections between LPA signaling and other receptor signaling networks. Answers to these questions and further insights into the control of lysophospholipid signaling will undoubtedly emerge in the not too distant future.

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