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# Mammary Tumorigenesis through LPA Receptor Signaling

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**Lysophosphatidic acid (LPA) is a lipid growth factor that is produced by an extracellular phospholipase, termed autotaxin (ATX), and acts via G protein-coupled receptors. In this issue of *Cancer Cell*, Liu et al. show that transgenic overexpression of ATX or LPA receptors leads to invasive and metastatic mammary cancer.**

Lysophosphatidic acid (LPA; mono-acyl-*sn*-glycero-3-phosphate) (Figure 1) is a bioactive phospholipid that stimulates the proliferation, migration, and survival of many cell types. Its "lyso" prefix notwithstanding, LPA has no membrane-perturbing effects but acts as a high-affinity ligand for specific G protein-coupled receptors. To date, there are six confirmed LPA receptors (termed $LPA_{1-6}$), which show a broad tissue distribution and have overlapping signaling properties (van Meeteren and Moolenaar, 2007). LPA signaling has been implicated in a great variety of biological processes, ranging from vascular development and neurite remodeling to inflammation and tumor progression. This multitude of activities may seem confusing but is consistent with the ubiquitous expression of LPA receptors and their coupling to a great diversity of G protein-mediated signaling pathways, including those initiated by Ras and Rho GTPases (van Meeteren and Moolenaar, 2007). The three classic LPA receptors, termed $LPA_{1-3}$, belong to the so-called EDG family of G protein-coupled receptors. The more recently identified LPA receptors, $LPA_{4-6}$, are related to the purinergic P2Y receptor family but are far distant from the EDG receptors, implying that LPA receptors have evolved from distinct ancestor genes.

LPA is produced extracellularly from more complex lysophospholipids, particularly lysophosphatidylcholine (LPC, the most abundant phospholipid in plasma), by a secreted (lyso)phospholipase D named autotaxin (ATX; also known as NPP2, nucleotide pyrophosphatase/phosphodiesterase 2), as illustrated in Figure 1 (van Meeteren and Moolenaar, 2007). ATX was originally identified as an "autocrine motility factor" for human melanoma cells, but its mode of action has remained elusive for a decade until it was discovered that ATX is identical to plasma lysophospholipase D, converting LPC to LPA. ATX is widely expressed, with highest mRNA levels detected in brain, lymph nodes, kidney, and testis; it is found overexpressed in various cancers. Gene targeting studies in mice indicate that ATX (encoded by *Enpp2*) is essential for vascular development, an unexpected finding given that none of the previous LPA receptor knockouts has hinted at a role for LPA in vasculogenesis (van Meeteren and Moolenaar, 2007).

Given its growth factor-like activities, it is not surprising that LPA has long been implicated in cancer. The oncogenic potential of the ATX-LPA receptor axis has become evident from studies in nude mice. In xenografted NIH 3T3 cells, overexpressed ATX cooperates with activated Ras to promote tumor aggressiveness and metastasis (Nam et al., 2000). Overexpression of $LPA_1$ in MDA-MB-231 breast carcinoma cells enhances tumor growth and promotes metastasis to bone (Boucharaba et al., 2004), whereas overexpression of $LPA_1$, $LPA_2$, and $LPA_4$ (but not $LPA_3$) in embryonic fibroblasts induces cell transformation and tumor formation in conjunction with MYC and TBX2 (Taghavi et al., 2008). Those studies also indicate that distinct oncogenic events must collaborate with ATX-LPA signaling to induce tumor formation.

Of the EDG family LPA receptors, $LPA_2$ provides the strongest case for a causal link to cancer. Not only is $LPA_2$ found overexpressed in various common cancers, including ovarian, colon, gastric, and invasive ductal breast carcinoma (Kitayama et al., 2004), but a recent study by Lin et al. (2009) demonstrates that

$LPA_2$ knockout mice have a marked decrease in tumor incidence and progression of chemically induced colon adenocarcinomas. Reduced colon tumorigenesis in the $LPA_2$-null animals correlated with reduced infiltration by macrophages (Lin et al., 2009), one of the predominant stromal cell types that contribute to tumor progression (Joyce and Pollard, 2009). Thus, it appears that $LPA_2$ is an important modulator of colon cancer. To determine the importance of $LPA_2$ in other types of cancer, one approach would be to crossbreed $LPA_2$-null mice with genetically engineered mouse tumor models, in which cancer is initiated through *Cre*-recombinase-mediated activation of oncogenic mutations in specific tissues (Jonkers and Berns, 2002).

Now, Liu et al. (2009) have taken a further step forward toward establishing a causal link between ATX-LPA receptor signaling and cancer progression. The authors established transgenic mouse models expressing ATX or each of the three classic LPA receptors ($LPA_{1-3}$) under the MMTV-LTR promoter. Strikingly, overexpression of either ATX or any of the LPA receptors resulted in mammary carcinomas with variable incidence and metastatic rates. Cancer onset was relatively late, with a latency ranging from 8 to 24 months. Major sites of metastasis were regional lymph nodes and lung, but not bone. Mammary glands from transgenic animals showed a high frequency and early onset of chronic mastitis, indicative of chronic inflammation prior to tumor development. The increased tumor development in the LPA receptor strains suggests that LPA is produced locally at levels sufficient to activate the transgenic LPA receptors. An alternative or additional possibility would be that forced overexpression renders LPA receptors constitutively active in a ligand-independent manner, although there is, as yet, no evidence to support this scenario. Determining local LPA levels in interstitial fluids would be very informative but is technically demanding, if not impossible.

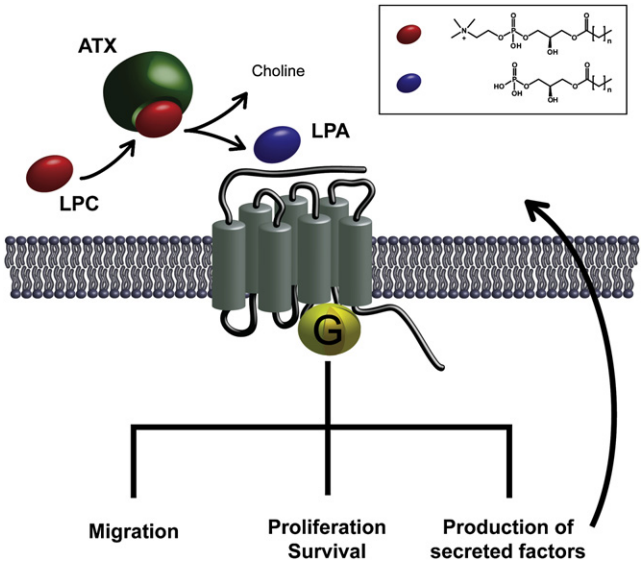


**Figure 1. ATX-LPA Receptor Signaling**
Autotaxin (ATX) functions as a lysophospholipase D that hydrolyzes extracellular lysophospholipids, in particular lysophosphatidylcholine (LPC), to produce bioactive LPA. LPC and LPA represent various species with varying acyl chains (inset). LPA acts on specific G protein-coupled receptors that signal through heterotrimeric G proteins (G) to alter cell behavior. LPA signaling leads not only to enhanced cell proliferation, migration, and survival, but also to modification of the cellular microenvironment via the production of secreted factors, including growth factors, chemokines, cytokines, and metalloproteases (Stortelers et al., 2008). LPA is susceptible to degradation through dephosphorylation by lipid phosphate phosphatases (not shown).

Comparative transcriptional profiling revealed that the mammary tumors induced by ATX/LPA receptor overexpression are heterogeneous, scattered throughout various transgenic mammary tumor models, supporting the notion that enforced ATX or LPA receptor expression in mammary glands allows accumulation of secondary transforming mutations leading to late-onset breast cancer development. Such a requirement for additional events is exemplified by the finding that overexpressed LPA receptors can transform fibroblasts only in conjunction with MYC overexpression and reduced p19ARF expression (Taghavi et al., 2008).

It is not immediately obvious why overexpression of ATX should lead to the same phenotype as LPA receptor overexpression, as observed by Liu et al. (2009). In general, increasing ligand concentration is not equivalent to increasing receptor expression levels. One plausible explanation is that ATX secreted by mammary epithelial cells may activate LPA-responsive adjacent cells, such as fibroblasts, leukocytes, and endothelial progenitors, to promote tumorigenesis. In fact, most solid tumors exploit nonmalignant stromal fibroblasts and myeloid cells to increase tumor growth and metastatic potential (Joyce and Pollard, 2009). In this respect, it is of note that LPA stimulates normal fibroblasts not only to proliferate and migrate, but also to produce many autocrine/paracrine mediators of tissue remodeling, inflammation, and tumor progression, notably growth factors, chemokines, cytokines, proangiogenic factors, and metalloproteases (Stortelers et al., 2008). Thus, mammary epithelium-derived ATX is likely to stimulate LPA signaling in nearby fibroblasts, which, in turn, results in the creation of a microenvironment that is permissive for tumor cell growth, invasion, and metastasis.

To what extent can the results of Liu et al. (2009) be translated to the human situation? The $LPA_2$ receptor is significantly overexpressed in patients with invasive ductal carcinoma, particularly in postmenopausal women (Kitayama et al., 2004). Because ATX is also expressed in ductal carcinoma (unpublished data), one can envision a scenario in which ATX and LPA act in an autocrine manner to stimulate the overexpressed $LPA_2$ receptor and thereby promote cancer progression. Although it remains to be seen whether continuous overexpression of either ATX or LPA receptors is required for tumor maintenance, the new findings reinforce the view that ATX and LPA receptors are attractive targets for therapeutic intervention. That ATX is an extracellular enzyme and G protein-coupled receptors, such as those for LPA, are highly "druggable" only adds to their attractiveness. The breast cancer models generated by Liu et al. (2009) and the colon cancer model of Lin et al. (2009) provide useful tools for developing therapeutics that target the ATX-LPA receptor axis.

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# Bringing H2AX into the Angiogenesis Family

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**The cell's ability to sense and respond to DNA damage is critical to maintain homeostasis and prevent the development of cancer. Paradoxically, Economopoulou et al. recently reported that a DNA damage response protein, H2AX, promotes tumor growth and angiogenesis.**

The DNA damage response pathway is crucial to maintaining genomic stability and cellular homeostasis. Toxic DNA lesions such as DNA double-strand breaks (DSBs) are frequently generated by endogenous sources, including the byproducts of metabolism, e.g., reactive oxygen species (ROS), spontaneous depurination of DNA strands, and replication fork collapse. Exogenous agents such as chemicals, ultraviolet radiation, and ionizing radiation also contribute to the formation of DSBs. Importantly, the inability to repair DSBs can result in genomic instability, cell death, and cancer (Fillingham et al., 2006).

Immediately following the generation of a DSB, a highly conserved DNA damage response pathway is activated to halt cell-cycle progression and repair the lesion. The molecular response to DNA damage begins with the recognition of the DSB followed by the activation of phosphatidyl inositol-3-kinase-like kinase family members ATM, ATR, and DNA-PK. Once activated, these kinases phosphorylate a number of effector molecules that regulate cell-cycle progression, DNA damage repair, and apoptosis. Histone H2AX is an important effector of the DNA damage response pathway that recruits DSB break recognition and repair proteins to the break (Bonner et al., 2008).

Consistent with a role for H2AX in DNA damage responses, recent studies have suggested that H2AX may function as a tumor suppressor. The chromosomal region (11q23) harboring H2AX is mutated or deleted in a variety of human cancers, including leukemia, breast, and head and neck cancers (Bonner et al., 2008). In addition, genetic inactivation of H2AX results in increased tumor burden in p53-deficient mice (Bassing et al., 2003).

Unexpectedly, a recent study in *Nature Medicine* has revealed a positive role for H2AX in tumorigenesis. Economopoulou et al. demonstrate that genetic inactivation of H2AX is sufficient to suppress tumor angiogenesis and growth in xenograft models (Economopoulou et al., 2009). Moreover, the authors demonstrate that specific inactivation of H2AX in endothelial cells similarly suppressed tumor angiogenesis and growth, indicating that H2AX and the DNA damage response in endothelial cells (ECs) play significant roles in tumor angiogenesis. Because hypoxia plays a critical role in the induction of tumor angiogenesis and has been previously shown to activate H2AX (Bencokova et al., 2009; Hammond et al., 2003), the authors examined the contribution of hypoxia to H2AX activation in ECs. In vitro studies revealed that hypoxia is sufficient to induce H2AX phosphorylation (γ-H2AX) and H2AX-dependent EC proliferation. Furthermore, the authors provide strong genetic data demonstrating a role for H2AX in additional models of hypoxia-induced neovascularization, including pathologic proliferative retinopathy and hind limb ischemia (Economopoulou et al., 2009). Together, these findings demonstrate that H2AX is an important component of hypoxia-induced angiogenesis and raise important questions regarding the mechanisms of H2AX-induced angiogenesis.

γ-H2AX may be induced in hypoxic ECs through replicative stress. Recent studies have identified hypoxia as a unique cellular stress that has the capacity to activate the DNA damage response pathway through damage-independent