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Regulation of tumor cell – Microenvironment interaction by the autotaxin-lysophosphatidic acid receptor axis

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Abstract

The lipid mediator lysophosphatidic acid (LPA) in biological fluids is primarily produced by cleavage of lysophospholipids by the lysophospholipase D enzyme Autotaxin (ATX). LPA has been identified and abundantly detected in the culture medium of various cancer cell types, tumor effusates, and ascites fluid of cancer patients. Our current understanding of the physiological role of LPA established its role in fundamental biological responses that include cell proliferation, metabolism, neuronal differentiation, angiogenesis, cell migration, hematopoiesis, inflammation, immunity, wound healing, regulation of cell excitability, and the promotion of cell survival by protecting against apoptotic death. These essential biological responses elicited by LPA are seemingly hijacked by cancer cells in many ways; transcriptional upregulation of ATX leading to increased LPA levels, enhanced expression of multiple LPA GPCR subtypes, and the downregulation of its metabolic breakdown. Recent studies have shown that overexpression of ATX and LPA GPCR can lead to malignant transformation, enhanced proliferation of cancer stem cells, increased invasion and metastasis, reprogramming of the tumor microenvironment and the metastatic niche, and development of resistance to chemo-, immuno-, and radiation-therapy of cancer. The fundamental role of LPA in cancer progression and the therapeutic inhibition of the ATX-LPA axis, although highly appealing, remains unexploited as drug development to these targets has not reached into the clinic yet. The purpose of this brief review is to highlight some unique signaling mechanisms engaged by the ATX-LPA axis and emphasize the therapeutic potential that lies in blocking the molecular targets of the LPA system.

Keywords

LPA; ENPP2; Invasion; Metastasis; Cancer stem cell; Therapy resistance

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jbior.2018.09.008>.

1. Introduction

The growth factor-like lipid mediator lysophosphatidic acid (LPA) activates many essential cellular responses that range from the regulation of cell proliferation, metabolism, motility, differentiation, and excitability to cell death (Benesch et al., 2018; Chun et al., 2018; Lee et al., 2018; Peyruchaud, 2009; Tigyi, 2010). In biological fluids the primary source of LPA is generated by the secreted lysophospholipase D designated autotaxin (ATX). Type I diacylglycerol kinases (DGKs), type II DGKs, and the type III DGK, which have substantial 2-monoacylglycerol kinase activity, can generate the sn-2 regioisomer of LPA contributing to intracellular pools of LPA (Sakane et al., 2018; Sato et al., 2016). LPA has multiple cellular targets but at the cell surface, the six G protein-coupled LPA receptors (LPAR) determine most of its cellular actions (Chun et al., 2018). LPA is inactivated by lipid phosphate phosphatase enzymes (LPP), which maintain low nanomolar levels of LPA in normal biological fluids (Leblanc et al., 2018; Leblanc and Peyruchaud, 2015). Research conducted on the ATX-LPAR-LPP axis has garnered a lot of attention in the field of oncology research because the molecular players of the ATX-LPAR axis have important effects on the growth, progression, metastasis and therapy resistance of cancers. The present review will cover three aspects of the ATX-LPA axis in malignancies by highlighting some of the reports we deemed are fundamental findings in the field. Thus, our objective is not to provide a comprehensive description of the many facets of LPA biology but instead to focus on the pivotal discoveries limited to oncology that have shaped our still rudimentary understanding of the ATX-LPAR axis in cancer.

2. The role of individual LPA receptors and ATX in tumor cells

After the original description of the mitogenic actions of LPA in non-transformed fibroblasts (van Corven et al., 1989, 1992), reports using cultured tumor cells have demonstrated that LPA can regulate the proliferation of transformed cells both as a mitogen (Xu et al., 1995a) or as an inhibitor of cell growth (i.e. anti-mitogen) (Tigyi et al., 1994). Whereas the mitogenic effect of LPA has been linked to pertussis toxin-sensitive G_i heterotrimeric G protein-mediated activation of the Ras – ERK1/2 signaling pathway (van Corven et al., 1993), inhibition of the growth of Sp2Age–O14 myeloma cells was accompanied by an elevation of cAMP level (Tigyi et al., 1994) in the cell, indicating a different mechanism of action and hinting that there might be multiple LPA receptors present in the different cell types that couple to distinct signaling pathways. Only after the molecular cloning and identification of six LPA GPCR (Kihara et al., 2014) has it become clear that LPA activation of LPA₁, LPA₂, LPA₃, and LPA₄ couple to the G_i —Ras-MAPK axis to promote cell proliferation, whereas LPA₅, predominantly expressed in Sp2Age–O14 myeloma cells, via G activates a Ca^{2+} -dependent adenylate cyclase that in turn inhibits cells growth. LPA GPCR identified and validated to date fall into two gene families. LPA₁, LPA₂ and LPA₃ belong to the Endothelial Differentiation Gene (EDG) family, whereas LPA₄, LPA₅, and LPA₆ represent a subcluster of the Purinergic GPCR family. The expression profile, signal transduction coupling and pharmacological ligands of these receptors can be accessed online in the IUPHAR/BPS Guide to Pharmacology (Chun et al., 2018). It is also important to note

that LPA has additional molecular targets that include the nuclear hormone receptor PPAR γ (Crowder et al., 2017; Tsukahara et al., 2006, 2010).

The LPA₁, LPA₂, and LPA₃ receptors when overexpressed using the mammary tumor virus promoter in transgenic mice cause malignant transformation and lead to the development of metastatic breast carcinomas (Liu et al., 2009). Likewise, overexpression of these individual LPA receptors in SKOV-3 human ovarian cancer cells drove malignant transformation and promoted tumor growth when injected into nude mice (Yu et al., 2008). In addition to the EDG family of LPAR, LPA₄ has also been shown to cause malignant transformation when overexpressed in mouse embryonic fibroblasts (MEF) overexpressing c-Myc and Tbx2 that are immortal but not transformed (Taghavi et al., 2008).

LPA₁ has been shown to regulate tumor cell motility and invasion (Li et al., 2009) in several different neoplastic cell types (Shida et al., 2004). This property of LPA₁ is likely related to its highly efficient coupling to the G_{12/13}-Rho-Rac-ROCK pathway (Chen et al., 2007; Ishii et al., 2000; Shida et al., 2004; Takuwa et al., 2002). It is well documented that LPA₁ mediates breast cancer metastasis particularly to the bone (Boucharaba et al., 2006, 2009; David et al., 2012, 2014b; Leblanc et al., 2014; Peyruchaud et al., 2003). However, this action of LPA is also influenced by stromal LPA₁ receptors in osteoclasts (David et al., 2014b; Leblanc et al., 2014; Peyruchaud et al., 2013). Tumor cell invasion and the production of matrix metalloproteinases are also affected by LPA₁ (Park et al., 2010; Shida et al., 2008).

LPA₂ has been linked to augmenting cell survival and rescuing apoptotically condemned cells from various noxious stimuli including radiation exposure (Deng et al., 2002, 2003, 2004, 2007, 2015). LPA₂ differs from other EDG LPAR in its C terminus where it contains the LIM domain and a C-terminal PDZ protein binding motif that promotes ligand-dependent interaction with several signaling proteins (Fig. 1). The LIM domain, which is a half Zn-finger domain mediates its binding to the proapoptotic protein Siva-1 (Lin et al., 2007). Siva-1 is activated by DNA damage and binds to Bcl-x_L, which destabilizes the mitochondrial outer membrane triggering the activation of the mitochondrial apoptosis pathway. LPA₂ inhibits the proapoptotic activity of Siva-1 by binding to it upon ligand activation and the macromolecular complex becomes polyubiquitinated and targeted for proteosomal degradation. Another LIM domain interacting protein is the thyroid receptor interacting protein-6 (TRIP6). TRIP6 is also a PDZ-binding protein and makes a ternary complex with the Na-H exchange response factor-2 (NHERF2) and the C-terminal PDZ motif of LPA₂ (E et al., 2009). Assembly of this ternary complex augments LPA₂-mediated activation of the pro-survival Akt and ERK1/2 kinase pathways (E et al., 2009). The TRIP6-NHERF2 and Siva-1 macromolecular complexes play an important non-G protein-dependent signal coupled to LPA₂ (Brindley et al., 2013). TRIP6 interaction with LPA₂ is not only important for inhibition of FAS-induced apoptosis but also for the promotion of cell migration (Lai et al., 2010). Although not yet shown in tumor cells, such macromolecular complex-mediated signaling is important for the spatial recruitment and coupling to LPA₂-interacting proteins that anchor the complex to the cytoskeleton. The interactome formed by LPA₂-NHERF2-phospholipase C β governs gradient sensing and directional migration in response to LPA in fibroblasts (Ren et al., 2014). In addition, LPA₂ has been reported to

promote cell migration via activation of c-src kinase and downstream tyrosine phosphorylation of villin in Caco₂ colon carcinoma cells. In these cells, LPA stimulated cell motility was insensitive to pertussis toxin, but was regulated by activation of PLC- γ 1 (Khurana et al., 2008). LPA₂ is also an integral player of the DNA damage response (DDR) pathway and is rapidly induced by γ -irradiation and the activation of NF κ B (Balogh et al., 2015; Kuo et al., 2018). We have shown that the transcription factor NF κ B mediates radiation-induced upregulation of LPA₂, which in turn augment the resolution of DNA double strand breaks indicated by the accelerated resolution of γ H2AX histone staining in wild type but not in LPA₂ knockout (KO) mice. In the context of physical interaction of LPA₂, it is noteworthy that a C-terminal-extended gain of function mutant has been described for this receptor in ovarian carcinoma cells (Huang et al., 2002). LPA₂ has been found to accelerate tumorigenesis in the colon in an azoxymethane and dextran sulfate sodium induced experimental model of colitis-associated cancer of mice (Lin et al., 2009) and also in mice carrying the ApcMin mutation (Lin et al., 2010). Additionally, LPA₂ KO mice showed reduced mucosal damage and fewer tumors than wild-type (WT) mice. Interestingly, in HCT116 and SW480 colon carcinoma cells, NHERF-2 promoted migration and invasion, whereas MAGI-3 inhibited these processes (Lee et al., 2011). MAGI-3 decreased the tumorigenic capacity of LPA₂ by reducing the activities of c-Jun N-terminal kinase and NF κ B.

LPA₃ was first reported to be a receptor that prefers sn-2 fatty acyl substituted LPA species and is highly sensitive to the presence of albumin in the LPA delivery vehicle (Bandoh et al., 1999; Hama et al., 2002; Heise et al., 2001). To what extent LPA₃ activation is affected by carrier proteins *in vivo* remains to be evaluated but LPA₃ undoubtedly functions in different tissue environments (Hama et al., 2007; Ye et al., 2011). In the context of neoplasia, siRNA to LPA₃ reduced proliferation, plasma membrane integrity and altered the morphology of A375 melanoma cells (Altman et al., 2010). Along with LPA₂, the LPA₃ GPCR has been shown to contribute to ovarian cancer aggressiveness (Yu et al., 2008). In particular, knockdown of the LPA₂ or LPA₃ receptors inhibited the production of IL-6, IL-8, and VEGF in SKOV-3 and OVCAR-3 cells (Yu et al., 2008). Although the crystal structure of LPA₃ has not yet been solved, homology models of this receptor have been reported and characterized (Fujiwara et al., 2005). For example, the src homology 3 ligand binding motif, R/K-X-X-V/P-X-X-P or (216)-KTNVLSP-(222), within the third intracellular loop of LPA₃ has been shown to be required for its prosurvival action (Jia et al., 2015). LPA₃ has been implicated in promoting pancreatic cancer progression, invasiveness and migration (Kato et al., 2012). LPA₃ has also been shown to regulate dendritic cell migration suggesting that the receptor may have a role in antitumor immunity (Chan et al., 2007).

LPA₄ (P2Y₉/GPR23) (Yanagida et al., 2009) has been linked to the regulation of cellular motility in nonmalignant (Lee et al., 2008) as well as malignantly transformed cells (Takahashi et al., 2017). Interestingly, LPA₄ when expressed in the SQ-20B cell line derived from head and neck squamous cell carcinoma reduced cell proliferation, motility, invasiveness (Matayoshi et al., 2013). The most profound phenotypic abnormality of LPA₄ KO mice in the C57/BL6 background is the impaired development of lymphatic vessels (Sumida et al., 2010). However, this phenotype is not observed in LPA₄ KO mice maintained on mixed 129/Sv and C57BL/6 background (Lee et al., 2008).

LPA₅ (GPR92) (Kotarsky et al., 2006; Pasternack et al., 2008) similarly to several other LPAR has been found to augment the progression of liver carcinoma cells that is associated with its aberrant DNA methylation (Okabe et al., 2011; Tsujino et al., 2010). LPA₅ acts as a chemorepellent in B16 mouse melanoma cells via receptor-mediated elevation of cAMP level (Jongsma et al., 2011). A unique action of LPA₅ is that it inhibits immune response by attenuating the activation of the T cell (Oda et al., 2013) and the B cell receptor (Hu et al., 2014). LPA₅-mediated inhibition of T cell receptor function attenuate tumor immunity and immune surveillance and might play a critical role in tumor progression *in vivo* (Oda et al., 2013).

The LPA₆ (P2Y5) receptor (Yanagida et al., 2009) is essential for human hair growth. Mutation of the *lpar6* gene causes woolly hair disorder (Kurban et al., 2013) and autosomal recessive hypotrichosis simplex (Nahum et al., 2011). In addition, LPA₆ has a role in telencephalon development (Geach et al., 2014). This receptor prefers 2-acyl-LPA rather than 1-acyl-LPA and couples primarily to the G12/13-Rho signaling pathway, making the structure-activity studies with this receptor more complicated (Inoue et al., 2012). In terms of cancer, LPA₆ exerts an inhibitory effect on pancreatic cancer invasiveness (Ishii et al., 2015), is uniquely upregulated in glioblastoma multiforme (Stangeland et al., 2015), and has been implicated in liver cancer, squamous cell carcinoma and metastatic prostate cancer (Younis and Rashid, 2017).

In biological fluids, LPA is generated by ATX, a lysophospholipase D that cleaves the choline/serine headgroup from lysophospholipids to generate LPA. ATX is a member of the ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP) enzyme family and is also known as ENPP2. ATX was originally identified as a secreted phosphatase in melanoma culture supernatant that promoted cancer cell motility (Stracke et al., 1992) but was later proven to function as a lysophospholipase D responsible for the production of LPA in blood (Tokumura et al., 2002; Umezu-Goto et al., 2002). ATX KO mice die in utero due to angiogenesis and neural tube defects (Tanaka et al., 2006; Fotopoulou et al., 2010 #154; van Meeteren et al., 2006).

Many cancers secrete ATX, which contributes to their invasive properties. Ovarian cancer cells in particular produce high levels of LPA (Baker et al., 2002; Sutphen et al., 2004; Xu et al., 1995b). ATX is not only secreted by tumor cells but also from cells of the tumor microenvironment (TME) (Gotoh et al., 2012; Lee et al., 2015), where it prepares the metastatic niche, promotes invasion, and inhibits antitumor immunity (Hu et al., 2014; Oda et al., 2013). Gene copy numbers are elevated in ovarian cancers in chromosomal region 8q24 (Fig. 2), which contains the gene encoding ATX (Dimova et al., 2006). Ectopic expression of ATX in mice has been shown to lead to mammary intraepithelial neoplasia, which subsequently develop into invasive and metastatic tumors (Liu et al., 2009).

ATX becomes overexpressed in patients with recurrent disease after treatment cycles with chemotherapy (Jazaeri et al., 2005). It is also the second most upregulated gene in therapy-resistant cancer stem cells (CSC) (Gupta et al., 2009) and is one of the 40 most upregulated genes in aggressively metastatic carcinomas (Euer et al., 2002). Moreover, a genome-wide siRNA screen identified ATX as a candidate drug-resistance gene in ovarian cancer (Vidot et

al., 2010). Indeed, studies have shown that the upregulated ATX-LPA₂ axis makes ovarian cancer cells resistant to Cisplatin and Adriamycin (E et al., 2009), and breast carcinomas resistant to Paclitaxel-induced apoptosis (Samadi et al., 2009). In addition, treatment of ovarian cancer cells with an inhibitor of ATX reversed the resistance to paclitaxel (Vidot et al., 2010).

ATX is upregulated as part of DNA damage repair and diminishes the efficacy of apoptosis-inducing therapies (Balogh et al., 2015). Multiple types of integrins have been shown to mediate the responses of LPA (Valenick and Schwarzbauer, 2006), and $\beta 3$ integrin has been reported to interact directly with ATX (Hausmann et al., 2011; Pamuklar et al., 2009). It has also been demonstrated that $\alpha 6\beta 4$ integrin signaling activates transcription factors of the NFAT family, which in turn regulate ATX expression (Chen and O'Connor, 2005). Thus, feed-forward LPA-induced upregulation of NFAT activity can, at least theoretically, upregulate the expression of ATX and LPA production. ATX is also upregulated by TNF α -mediated activation of NF κ B (Balogh et al., 2015). NF κ B-dependent transcriptional regulation of ATX is particularly important in the context of the inflammatory milieu of the TME where several cytokines such as interleukin 6, interleukin 8, vascular endothelial growth factor, and urokinase plasminogen activator (Fang et al., 2004; Hu et al., 2001; Pustilnik et al., 1999; Schwartz et al., 2001) can potentially drive overexpression of ATX and the ensuing LPA production. Taken together, ATX provides all LPAR with their cognate ligand and is a target of gene duplication as well as transcriptional upregulation by a host of factors in the TME.

3. The role of the ATX-LPA receptor axis in cancer stem cells

The genetic instability of tumor cells leads to heterogeneity in a cancer cell population, be it of the primary or metastatic tumors. However, there is yet another type of heterogeneity that distinguishes cancer stem cells (CSC) from their daughter cells due to the unique properties of CSC in self-renewal, invasion, metastasis, and engraftment in distant tissues. Thus, even though cancer may start from a single cell that undergoes malignant transformation, the tumors that develop cannot be viewed as a single lineage. This property of malignancies represents significant challenges to cancer therapy (Prasetyanti and Medema, 2017). Along with recent advances in the successful eradication of primary tumors, more attention is being given to therapies that target CSC and cancer metastasis. An important mechanism in the development of metastatic lesions is the interconnected relationship between the invading CSC and the pre-metastatic niche. Recent studies suggest an important role for the ATX-LPAR signaling axis in CSC growth, invasion, metastasis and the reprogramming of the TME. There are many challenges in therapeutically targeting CSC, due to their rarity, dormancy, and resistance to therapy attributed in part by intrinsic properties of the CSC and the protective environment of the TME. There is increasing evidence that the ATX-LPA axis is involved in all of these mechanisms.

LPA has been recognized for its ability to promote epithelial-mesenchymal transition (EMT), which is a key step in the development of the invasive and metastatic ability of CSC (Burkhalter et al., 2015; Ha et al., 2016; Harma et al., 2012; Hashimoto et al., 2016; Jahn et al., 2012; Jiang et al., 2011; Ray et al., 2017; Xu et al., 2017). ATX upregulation has been

recognized as an event accompanying EMT (Castellana et al., 2012; Dai et al., 2015; Ptaszynska et al., 2010). In particular, ATX-LPA has been reported to activate signaling pathways driving EMT such as the PI3K/AKT/mTOR/Skp2/p27 (Xu et al., 2017), p53 (Jiang et al., 2011), NFAT-1 (Dai et al., 2015), Transcriptional co-activator with PDZ-binding motif (TAZ) (Jeong et al., 2013), Kruppel-like factor 4 (KLF4) (Shin et al., 2014), and NFkB (Tobar et al., 2010).

In epithelial ovarian cancer cells, the ATX-LPA₁ receptor axis has recently been recognized as a mechanism responsible for driving the expression of CSC-associated genes such as OCT4, SOX2, and aldehyde dehydrogenase 1 (Seo et al., 2016). Silencing LPA₁, ATX or AKT1 prevented the upregulation of these markers in ovarian CSC (Seo et al., 2016). We showed that ATX inhibitors dose-dependently reduce the viability of 4T1 murine breast carcinoma-derived mammospheres (Banerjee et al., 2017). We have tested the effect of ATX inhibitors BMP-22 (Gupte et al., 2011) and BESA-3 (Banerjee et al., 2017) and the LPA₂ antagonist compound 35 reported by Beck et al. from Amgen Inc., on the growth and viability of CSC mammosphere cultures established from 4T1 cells (Fig. 3). Both ATX inhibitors significantly reduced the growth and viability of 4T1 mammospheres. The LPA₂ antagonist was most effective in reducing the growth and viability of the cultured mammospheres suggesting that this receptor subtype may have a profound role in the growth of cultured 4T1 CSC. Taken together, the ATX-LPA axis emerges as a major regulatory system of CSC growth and progression (Lee et al., 2018).

4. The role of ATX and LPA in the tumor microenvironment

Apart from cancer cells, the TME is an equally important source of ATX and LPA. Compelling evidence for the role of stromal ATX and LPA₁ in the bone metastasis of breast carcinoma has been provided by the Peyruchaud group (Boucharaba et al., 2004, 2006; David et al., 2014a, 2014b; Leblanc et al., 2014, 2018). Using MDA-B02, an ATX non-expressing variant of the breast carcinoma cell line MDA-MB-231, they have shown that treatment of mice either with the LPA_{1/3} inhibitor Ki16425 or the ATX inhibitor BMP-22 greatly reduced the number of bone metastases. The number of micrometastatic colonies from the bone marrow of BMP-22 treated mice showed a 93% reduction and the growth of already established osteolytic metastases were attenuated by 43% when compared to controls (Leblanc et al., 2014). Gotoh et al. demonstrated that the ATX inhibitor BMP-22 reduced the number of lung metastasis of B16eF10 melanoma (Gotoh et al., 2012). Lee et al. using the LPA₁, LPA₂ and LPA₅ KO mice on the C57/BL6 genetic background syngeneic with the B16-F10 melanoma found that the number of metastatic nodules was reduced by approximately 50% in the LPA₁ KO mice and almost completely absent in LPA₅ KO host mice. They also found a similar decrease in the number of fluorescently-labeled B16-F10 cells attaching to the lungs of LPA₁ and LPA₅ KO mice, respectively 24 h after inoculation via the tail vein (Lee et al., 2015). Treatment of the mice with either the LPA_{1/3} antagonist Ki16425 or the ATX inhibitor BMP-22 alone partially reduced the number of lung metastases by B16eF10 whereas, combined treatment caused near 90% reduction in lung metastases. Because B16eF10 cells do not express LPA₁ and LPA₃ transcripts, this observation further emphasizes the importance of stromal LPAR in the seeding of metastasis (Lee et al., 2015). With regard to stromal ATX, adipocytes are a rich source (van Meeteren et

al., 2006). The Brindley group has elegantly demonstrated that adipocyte-rich stroma of the mammary gland provide an abundant source of LPA for the growth and progression of breast carcinomas (Benesch et al., 2015a, 2015b; Gaetano et al., 2009; Samadi et al., 2011; Tang et al., 2015; Venkatraman et al., 2015). In addition, it is also important to recognize that the ATX-LPA axis is responsible for the production of a host of proinflammatory mediators from stromal elements in the TME that have a profound role in the progression and metastatic spread of carcinomas (Benesch et al., 2018).

The ability of cancer to develop resistance to therapy is a major health problem which was identified recently by the NCI Cancer Moonshot initiative as a priority research topic for the scientific community. A critical barrier to progress in dealing with the problem of cancer resistance to therapy is the lack of drugs that reverse resistance in the tumor cell itself and also block the supportive actions of the TME. Recent research began addressing this need by identifying the ATX-LPA axis as a major system that regulates therapy resistance in various cancers. As we have described in an earlier section of this paper, ATX is the second most upregulated gene in therapy-resistant CSC, whereas the LPA inactivating enzyme lipid phosphate phosphatase (LPP) is the most downregulated gene (Gupta et al., 2009). As mentioned above, ATX becomes overexpressed in patients undergoing treatment with repeated cycles of chemotherapy (Jazaeri et al., 2005) and in a genome-wide siRNA screen ATX has been identified as candidate drug-resistance gene in ovarian cancer (Lee et al., 2018; Vidot et al., 2010). LPA has been reported to activate Arf6 and enhance invasion and drug resistance of renal carcinoma cells (Hashimoto et al., 2016; Yamauchi et al., 2017). ATX and LPA₂ are upregulated as part of DNA damage repair and diminish the efficacy of apoptosis-inducing therapies. These observations lead to a novel hypothesis which predicts that when DNA damage repair goes awry it will transcriptionally and functionally upregulate the ATX-LPA₂ signaling axis leading to therapy resistance (Fig. 4).

LPA₂ has been demonstrated to protect cells from apoptosis elicited by exposure to ionizing radiation and chemotherapeutics (Fig. 5) (Deng et al., 2002; Deng et al., 2004; Deng et al., 2003; E et al., 2009; Kuo et al., 2018). We demonstrated that the ATX and LPA₂ genes are induced by exposure to ionizing radiation (Balogh et al., 2015; Kuo et al., 2018). Specifically, the upregulation of LPA₂ is downstream of the DDR as CGK-733, an ATM/ATR kinase inhibitor, blocked radiation-induced upregulation of lpa2 transcripts in IEC-6 cells (Balogh et al., 2015). We found that stimulation of the LPA₂ receptor causes a sustained activation of the ERK1/2 and Akt kinases lasting more than 12 h (unpublished). These two kinases play key roles in the regulation of cell survival by inhibiting apoptosis. The central role of the Akt kinase–NFκB transcription factor is well documented in mediating resistance to radiation and chemotherapy (Oeck et al., 2017; Szymonowicz et al., 2018). We hypothesize that the upregulated ATX-LPA₂ receptor axis via the IEX-1 scaffold protein (de Laval et al., 2014) and ERK1/2 are important amplifiers of the activity of Akt–NFκB.

In particular, the uniquely long-lasting activation of LPA₂-mediated antiapoptotic signal is mediated by an interactome, which inhibits protein phosphatase 2a (PP2A) – the key terminator of Akt and ERK1/2 phosphorylation (Garcia et al., 2002; Rocher et al., 2007). A key scaffold protein that brings together this interactome is the immediate early gene IEX-1.

IEX-1 expression is driven by ATM kinase, a critical enzyme in the DDR pathway (Pawlikowska et al., 2010). We suggest that LPA₂ activation of ERK1/2 via heterotrimeric G_i protein and MEK kinase, leads to the downstream phosphorylation of IEX-1 by ERK1/2, which stabilizes the complex and promotes IEX-1 association with the B56 subunit of PP2A (de Laval et al., 2014). This association allows for ERK1/2 to phosphorylate the B56 subunit of PP2A that triggers its dissociation from the PP2A holoenzyme (Letourneux et al., 2006). PP2A is constitutively active in cells and functions to inhibit ERK1/2 and AKT. We hypothesize that the reduced PP2A activity, as a result of ERK1/2-IEX-1-mediated dissociation of B56 subunit, will give rise to a prolonged activation state of ERK1/2 and Akt, which enhance cell survival and counteract therapeutically-induced apoptosis in neoplastic cells. We are currently testing this hypothesis (Fig. 5). Initial data reinforce the protective action of LPA against frontline chemotherapeutics like Gemcitabine (Fig. 5A) and that exposure of 4T1 breast CSC to either 24 Gy fractionated γ -irradiation or 80 nM Paclitaxel treatment causes transcriptional upregulation of LPA₂ and ATX (Fig. 5 B & C). We are hopeful that drugs that inhibit LPA₂ and ATX can be used as adjuvants to restore the sensitivity of CSC to radiation- and chemotherapy as we have reported for a group of ATX inhibitors (Banerjee et al., 2017). We further demonstrate in Fig. 5D that BMP-22 reduced the IC₅₀ of Paclitaxel in 4T1 cancer cells by an order of magnitude.

As more proof of principle studies will demonstrate the utility of drug candidates that block the ATX-LPAR targets in different malignancies, it is conceivable that companies with compounds already at hand will launch clinical trials for the experimental treatment of cancer patients.

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Abbreviations:

ATX	Autotaxin
BESA-3	utotaxin inhibitor benzene sulfonamide compound 3
BMP-22	Autotaxin inhibitor benzyl methyl phosphonic acid compound 22
CSC	Cancer stem-like cell
EDG	Endothelial Differentiation Gene
ENPP	ecto-nucleotide pyrophosphatase/phosphodiesterase
LPA	lysophosphatidic acid
LPAR	LPA G protein-coupled receptor
NHERF-2	NaH exchange response factor-2

TRIP6 thyroid receptor interacting protein-6

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Fig. 1.
Anti-apoptotic signaling pathways activated by LPA₂.

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Fig. 2. A cross-cancer query of *enpp2*, the gene encoding ATX using the cBio Cancer Genomics Portal. ATX (encoded by *enpp2* gene) is amplified or upregulated predominantly in ovarian cancer. Datasets obtained from The Cancer Genome Atlas.

Fig. 3.

LPA2 inhibition disrupts spheroid formation in 4T1 mouse mammary carcinoma cells. 4T1 cells were plated in triplicate in ultra-low adherence 24-well plates at a density of 7.5×10^3 cells per well in spheroid formation medium (RPMI with 200 μ M L-glutamine, 100 U penicillin, 100 μ g/ml streptomycin, 1 $\times$ N-2 supplement, 20 ng/ml mouse EGF and 20 ng/ml mouse FGF). Cells were incubated at 37 °C with 5% CO₂ for 4 days to allow spheroid formation, then treated with a range of 0–3 μ M Amgen 35 (LPA₂ antagonist), BMP-22 (ATX inhibitor) or BESA-3 (ATX/LPA₁ inhibitor) and incubated an additional 3 days. Cells were surveyed under 100 $\times$ magnification (microscopy panels), and spheroids were manually counted, plotted versus Amgen 35 concentration and GraphPad Prism v. 5.0a was used to perform non-linear regression in a variable slope model to determine the IC_{50} of Amgen 35 for spheroid formation (dose-response panel). Finally, cell viability was determined using Promega CellTiter Blue reagent as per manufacturer's instructions. Briefly, 50 μ l CellTiter Blue were added to each well, and plates were incubated at 37 °C for 3 h prior to measuring fluorescence at excitation/emission wavelengths of 560/590 nm, respectively. Relative fluorescence was then background-corrected and normalized to percentage vehicle signal for each compound tested (bar graph panel). GraphPad Prism was used to perform one-factor ANOVA with Bonferroni's post-test to determine whether cell viability differed significantly between vehicle and drug treatment (** = $p < 0.01$, *** = $p < 0.001$; $n = 3$).

Fig. 4.
When DNA damage repair goes awry it can lead to therapy resistance. A new hypothesis.

Fig. 5.

ATX and LPA₂ play key roles in resistance against radiation-induced programmed cell death. (A) LPA protects HPAF II human pancreatic cancer cells against gemcitabine-induced apoptosis. HPAF II cells were cultured in growth media (DMEM F12 supplemented with heat inactivated 10% FBS, 200 μM L-glutamine, 100 U penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin). Next day, cells were serum starved for 24 h followed by exposure to 10 μM Gemcitabine with or without LPA (at 3 μM or 10 μM). Caspase 3/7 activity was measured after 24 h of treatment using the CaspaseGlo[®] Kit (Promega). (B) LPA₂ and (C) ATX expression are upregulated in 4T1 CSC upon exposure to Paclitaxel or γ -irradiation. 4T1 CSC were generated as described in Fig. 3 and spheroids were allowed to form for 3 days. At day 4, CSCs were either treated with 80 nM of Paclitaxel (PCTXL) or fractionated doses of γ -irradiation (6Gy/day for 4 days). CSCs were harvested for QPCR analysis after 4 days of PCTXL treatment or 24 h after the last dose of fractionated radiation. GraphPad Prism was used to perform one-factor ANOVA with Bonferroni's post-test to determine the significance in LPA₂ or ATX expression in vehicle versus PCTXL or radiation treatment, respectively (***) = $p < 0.001$; $n = 3$). (D) The ATX inhibitor BMP-22 enhances 4T1 cell killing by PCTXL. 4T1 cells were plated in quadruplicate in 96-well plates at a density of 5×10^3 cells per well in RPMI with 1% (v/v) charcoal-stripped FBS and incubated overnight at 37 °C with 5% CO₂. Cells were then treated for 24 h with 3 μM BMP-22 followed by 48 h treatment with a dose range of 0–1 μM PT in RPMI with 1% (v/v) charcoal-stripped FBS. After 48 h incubation, cells were allowed to recover in RPMI with 1% (v/v) charcoal-stripped FBS for an additional 72 h. Viability was then assessed via Promega CellTiter Blue

(as per manufacturer's instructions) and normalized to % vehicle control samples. Data were plotted using GraphPad Prism v. 5.0a, and non-linear regression analysis was performed in a variable slope model in order to determine LD₅₀ concentrations for PCTXL in the presence and absence of BMP-22.

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