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Review

Complexities of lysophospholipid signalling in glioblastoma

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ABSTRACT

Glioblastoma multiforme (GBM) is the most malignant brain tumour and continues to have a very poor median survival of 12–16 months despite current best therapies. These aggressive tumours always recur after treatment and are defined by their ability to diffusely infiltrate and invade normal brain parenchyma. Autotaxin is overexpressed in GBM, and is a potent chemotactic enzyme that produces lysophosphatidic acid. Lysophospholipid (LPL) signalling is known to increase invasion of solid tumours and is also dysregulated in GBM. The LPL pathway has been shown to interact with known cancer-related signalling pathways, including those for epidermal growth factor and yes-associated protein, which are also dysregulated in GBM. The interactions between these pathways provide insights into the complexities of cancer signalling and suggest potential novel targets for GBM.

1. Introduction

Glioblastoma multiforme (GBM) is the most malignant (World Health Organization grade IV) glioma and continues to have a very poor median survival of 12–16 months despite current best therapies (maximal safe resection with concurrent temozolomide chemotherapy and radiation therapy) [1]. These aggressive tumours always recur after treatment and are defined by their ability to diffusely infiltrate and invade normal brain parenchyma. Thus the search for targeted agents inhibiting cell proliferation, survival and invasion has intensified. Research into the epidermal growth factor receptor (EGFR) pathway has led to clinical trials of EGFR and phosphatidylinositol-3-kinase (PI3K) inhibitors that modulate cell survival and proliferation in pre-clinical models. However, results from early EGFR inhibitor trials have not delivered on their promise and PI3K inhibitor trials are ongoing [2–4]. These trials have made it obvious that strategies combining therapies against multiple targets are required to account for the existence of complex pathway interactions and redundancies. For example, matrix metalloproteases (MMP) degrade extracellular matrix components to produce more favourable conditions for cell migration and invasion [5]. However, they also cleave and activate growth factors such as epidermal growth factor (EGF) [6]. The function of integrins and their influence on cell morphology and migration has also been enlightening [7,8]. More recently, other promising factors have been identified, including autocrine motility factor receptor, heparin-binding epidermal growth factor, ephrin-B3, netrin 4 and autotaxin (ATX) [9]. The latter three are of interest because their role in cell migration and motility in neural stem cells suggests a similar role in glioma-derived cancer stem cells, that have a putative role in GBM progression [10,11]. ATX in particular is a powerful chemotactic enzyme involved in lysophospholipid (LPL) signalling, and its recent prominence in the literature has highlighted the importance of lipid signalling within complex intracellular pathway interactions. This review focuses on the role that LPL signalling may play in gliomagenesis and its potential as a target in the treatment of this highly malignant disease.

2. Lysophosphatidic acid

Lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) are the main membrane-derived lipid signalling molecules. LPA has a 3-carbon glycerol backbone, with an attached single acyl or alkyl chain of varying length which imparts some differences in receptor efficacy [12]. Whilst some LPA production may occur

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intracellularly, much of it is produced extracellularly by secreted enzymes. There are three known pathways: (1) cleavage of LPL (such as lysophosphatidylcholine) by lysophospholipase D, (2) deacylation of phosphatidic acid by phospholipase A1 and A2, and (3) mild oxidation of low-density lipoprotein (non-enzymatic).

Lysophospholipase D is now more commonly known as ATX which derives its name from early characterisation of its stimulatory effect on melanoma cell motility [13–15]. It is also known as ectonucleotide pyrophosphatase and phosphodiesterase-2 (ENPP-2) and is part of the family of ENPP enzymes which are traditionally known for their involvement in nucleotide metabolism [13]. LPA production is mainly via ATX catalysis. This is confirmed by the uniformly fatal outcome of homozygous ATX ($Atx^{-/-}$) knockout mice and the 50% reduction in circulating plasma LPA levels in embryos with heterozygous ATX ($Atx^{+/-}$) expression [16,17].

The phospholipase A enzymes play an important role in determining the position of the acylation of the phosphoglycerol backbone and so, whilst there is currently no evidence of its upregulation, it may still be an important pathway in pathological states, as it may influence the action of liberated LPA species by playing a role in determining the dominant LPA species produced (acyl or alkyl) [18].

Physiologic LPA is present in small amounts in tissues since it regulates its own production via negative feedback inhibition of its main synthesising enzyme, ATX [19]. Thus, the influence of this signalling pathway is largely autocrinic/paracrinic [12–14]. Further, circulating platelets and erythrocytes secrete LPA which is then bound to albumin to protect it from rapid enzymatic degradation by lysophospholipases, lipid phosphate phosphatases and LPA acyl transferases [12]. Cancer cells can produce increased amounts of ATX/LPA and it has been previously reported that some malignant effusions (such as those of ovarian cancer) have elevated levels of LPA compared to other malignancies [2,20,21]. In addition, circulating plasma levels of LPA can be elevated in malignancy and this may be the result of elevated production (induction of ATX) [14].

3. LPA receptors

Only a brief review of the LPA receptors will be provided here as there are many other detailed reviews available [12,16,22–24]. At the time of writing, the International Union of Basic and Clinical Pharmacology had recognised six definitive G-protein coupled-LPA receptors (collectively LPAR) designated LPA_{1-6} (Table 1). Broadly, the receptors fall into two families: endothelial differentiation gene (Edg) and non-Edg (purinergic) receptors [12]. LPA_1 (Edg2), LPA_2 (Edg4) and LPA_3 (Edg7) are members of the Edg family and are the best characterised to our knowledge with LPA_1 being the dominant LPA receptor in the central nervous system (CNS) [13,23,25]. LPA_1 is coupled to the G-proteins, $G_{i/o}$, G_q and $G_{12/13}$, which allows it to signal via multiple pathways, including major cancer-related pathways such as mitogen-activated protein kinase (MAPK), Akt/PKB, and small GTPases such as Rho/ROCK [12,26]. LPA_1-induced PI3K signalling (which activates Akt/PKB) via the p110β/γ subunits (of PI3K) has also been reported [27–30]. Whilst LPA_2 is expressed in embryonic brain it has little to no expression in the adult CNS [31]. LPA_3 is expressed in the brain, but unlike LPA_1 and LPA_2 it is coupled to the G-proteins, $G_{i/o}$ and G_q, but not $G_{12/13}$ and so is less responsive to LPL than LPA_1 [27]. As LPA_3 does not signal via $G_{12/13}$, it is not involved in changes in cell morphology, and therefore unlikely to be involved in cell migration [27].

The non-Edg or purinergic family of LPA receptors are genetically distinct from the original Edg family of LPA receptors [23]. Importantly, this results in the non-Edg family having an increased affinity for alkyl-LPA species, as opposed to the Edg family having increased affinity for the acyl variants [22,23]. Current members of the non-Edg family include LPA_4 (P_2Y_9), LPA_5 (GPR92) and LPA_6 (P_2Y_5). LPA_4 signals via G_s, G_q and $G_{12/13}$-proteins and probably plays a role in cell motility and migration [23]. Mouse embryonic fibroblasts (MEF) derived from *Lpa4* (null) knockout mice have been reported to exhibit hypersensitivity to LPA induced motility. These MEF had increased levels of phosphorylated Akt (pAkt) when stimulated by LPA. The elevated pAkt levels and associated motile response were attenuated when *Lpa4* was reintroduced into the cells, suggesting that LPA_4 has an action which might suppress signalling activity of the LPA_1 receptor [32]. LPA_5 (GPR92) and LPA_6 (P2Y5) were both discovered subsequent to LPA_4 and to our knowledge there is no reported role for LPA_5 in tumourigenesis. LPA_6 however, may cause morphological changes in vascular endothelial cells and therefore may play a minor or indirect role in gliomagenesis via effects on vascular development [22,23,27].

LPL have a wide range of physiological and pathological effects owing to the myriad G-proteins they are coupled to. As a result, they modulate numerous normal physiologic processes, including cell proliferation and apoptosis, cell differentiation, cell adhesion and migration, cell morphology (including neurite retraction and synaptic cleft shape modulation), normal CNS development and autoimmunity [12,13,16,18,25,33–35]. Dysregulation of these events is strongly implicated in tumourigenesis.

4. LPA signalling in cancer

Since Stracke et al. discovered the promotile effects of ATX on melanoma cells in 1992, the LPA pathway has been investigated

Table 1
LPA receptors and their relevance to cancer research

LPA receptor		Coupled G-proteins	Relevance to cancer research	Reference
Edg	LPA1	Gi/o, Gq, G12/13	– Dominant LPA receptor expressed in adult CNS – Increased expression in cancers including GBM – Linked to cell proliferation, survival, migration/invasion	Kishi et al. [2]
	LPA2	Gi/o, Gq, G12/13	– Not present in adult CNS – Not markedly elevated in GBM	Kishi et al. [2]
	LPA3	Gi/o, Gq	– Expressed in adult CNS – Not markedly elevated in GBM	Kishi et al. [2], Choi et al. [16]
Non-Edg	LPA4	Gs, Gq, G12/13	– Activation of LPA4 has been shown to be functionally antagonistic (anti-migration, anti-proliferation) to LPA1	Kato [59], Yanagida and Ishii [23], Lee et al. [32]
	LPA5	Gq, G12/13 Increase in cAMP not shown to be Gs-mediated	– Activation has been shown to reduce cell migration	Jongsma et al. [54]
	LPA6	G12/13, (possible role for Gs, Gi)	– Possible similar function to LPA4 – May regulate vascular permeability	Yanagida and Ishii [23]

cAMP = cyclic adenosine monophosphate, CNS = central nervous system, Edg = endothelial differentiation gene, GBM = glioblastoma multiforme, LPA = lysophosphatidic acid.