Critical role of acylglycerol kinase in epidermal growth factor-induced mitogenesis of prostate cancer cells

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Abstract
The bioactive phospholipids, LPA (lysophosphatidic acid) and PA (phosphatidic acid), regulate pivotal processes related to the pathogenesis of cancer. Recently, we cloned a novel type of lipid kinase that phosphorylates monoacylglycerols (such as 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand) and diacylglycerols, to form LPA and PA, respectively. This AGK (acylglycerol kinase) is highly expressed in prostate cancer cell lines and the results reviewed here suggest that AGK might be a critical player in the initiation and progression of prostate cancer. Intriguingly, down-regulation of endogenous AGK inhibited EGF (epidermal growth factor), but not LPA-induced ERK1/2 (extracellular-signal-regulated kinase 1/2) activation and progression through the S-phase of the cell cycle. In this review, we will summarize the evidence demonstrating that AGK amplifies EGF growth signalling pathways that play an important role in the pathophysiology of prostate cancer. Because LPA has long been implicated as an autocrine and paracrine growth stimulatory factor for prostate cancer cells, the identification of this novel lipid kinase that regulates its production could provide new and useful targets for preventive or therapeutic measures.

LPA (lysophosphatidic acid): a potent mitogen for prostate cancer
For over 50 years, the primary form of therapy for advanced prostate cancer has targeted the androgen receptor. However, there have been few cures and virtually all patients with metastatic prostate cancer treated with androgen deprivation will progress to androgen independence. However, it is still not fully understood why prostate cancer cells eventually become androgen independent, resistant to therapy, and ultimately cause the death of the patient. The most likely way to develop new and effective therapies for prostate cancer is to improve our understanding of the processes leading to the initiation and progression of this disease. Other factors responsible for the initiation and progression of prostate cancer remain poorly understood, although growth factors, such as EGF (epidermal growth factor) and IGF-II (insulin-like growth factor-II), and hormones have been implicated in the growth and survival of prostate cancer cells [1].

A recent addition to the ranks of prostate mitogenic factors is LPA, a major mitogen in serum. Indeed, the ability of serum to stimulate cell growth appears to be mediated to a large extent by LPA [2–4], and the related lysoosphospholipid, S1P (sphingosine 1-phosphate), which has a similar structure to LPA but with a sphingosine rather than glycerol backbone, and whose importance in cell growth and survival was discovered in our laboratory [5]. Both LPA and S1P regulate an array of cellular processes related to pathogenesis of cancer, especially prostate and ovarian cancers, including initiation and regulation of proliferation, enhancement of survival, suppression of apoptosis, cytoskeleton reorganization and tumour cell motility and invasiveness [2,3,5]. LPA is a potent mitogen for PC-3 prostate cancer cells, which are androgen independent and models for more advanced carcinomas [6]. Growth and signalling patterns in these cells in response to serum resemble those mediated by LPA and are dependent on G1 βγ subunits, suggesting an important regulatory role for LPA in the growth of prostate cancer cells [7]. It has also been suggested that LPA responsiveness might be enhanced in more advanced carcinoma [8].

LPA as a ligand for a new class of GPCRs (G-protein-coupled receptors)
Progress in understanding LPA actions was accelerated by the discovery that it is a ligand of several GPCRs [9]. To date, there are three established LPA receptors, LPA1, LPA2 and LPA3 [4,9,10], which are coupled to a variety of G-proteins and regulate diverse cellular responses [4,10]. Expression of LPA receptors correlates with more advanced prostate cancer cell lines [8] and LPA2 and LPA3 are aberrantly expressed in ovarian cancer cells [11,12], indicating their potential role in the pathophysiology of cancer. Recently, a fourth putative LPA receptor was described (LPA4/GPR23/P2Y9) which is distinct from the other LPA receptors [13]. LPA also has

Key words: acylglycerol kinase, epidermal growth factor (EGF), G-protein-coupled receptor (GPCR), lysophosphatidic acid (LPA), phosphatidic acid (PA), prostate cancer.

Abbreviations used: AGK, acylglycerol kinase; EGF, epidermal growth factor; EGFR, EGF receptor; ER, endoplasmic reticulum; ERK, extracellular-signal-regulated kinase; GPCR, G-protein-coupled receptor; LPA, lysophosphatidic acid; mTOR, mammalian target of rapamycin; PA, phosphatidic acid; PPAR-γ, peroxisome proliferator-activated receptor-γ; PTX, pertussis toxin; S1P, sphingosine 1-phosphate.

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a novel intracellular function as a high-affinity ligand for PPAR-\(\gamma\) (peroxisome proliferator-activated receptor-\(\gamma\)), a transcription factor that regulates genes controlling energy metabolism [14] and can exacerbate mammary gland tumour development [15].

**How does LPA so profoundly influence proliferation of so many types of cells including prostate cancer cells?**

LPA can stimulate cell proliferation by several interactive mechanisms: (i) LPA enhances SRE (serum response element) activity in the promoters of immediate early growth-related genes [16]; (ii) LPA stimulates secretion of polypeptide growth factors, such as EGF and IGF-II [17]; (iii) LPA enhances survival and suppresses apoptosis by reducing levels of the apoptosis-promoting protein Bax [18]; (iv) LPA can also sensitize some types of cells to the growth promoting effects of polypeptide growth factors; and (v) finally, LPA can stimulate EGFR (EGF receptor) transactivation by enhancing metalloproteinase activity and processing of proHB-EGF to EGF [19]. Thus, in addition to actions through conventional GPCR signalling pathways, LPA receptors can indirectly regulate cell functions by transactivating the EGF tyrosine kinase receptor [19–21]. This cross-communication between different signalling systems is not only important for the growth promoting activity of LPA [20,21], it may also be a clue to its pathophysiological role in prostate cancer [19], head and neck squamous cell carcinoma [22], and kidney and bladder cancer [23].

**LPA and signal transduction**

Downstream links involving LPA to growth and survival are complex and to some extent are reminiscent of mitogenic signalling by other GPCRs. Most studies indicate that LPA\(\_1\) and LPA\(\_2\) can couple to the G\(\alpha_{16}\), G\(\alpha_{12/13}\) and G\(\gamma\) families, whereas it appears that although LPA\(\_1\) is also linked to activation of G\(\alpha_{16}\) and G\(\gamma\), it does not couple efficiently with G\(\alpha_{12/13}\). LPA-induced activation of G\(\alpha\), which is sensitive to PTX (pertussis toxin), leads to decreased cAMP, activation of the ERK (extracellular-signal-regulated kinase) cascade, and potentially tyrosine phosphorylation and proliferation. G\(\alpha\), as well as G\(\alpha\), can link to phospholipase C, whereas LPA-induced activation of G\(\alpha_{12/13}\), which directly binds to Rho guanine nucleotide exchange factor and activates the small GTPase Rho, leads to cytoskeletal rearrangements and changes in cell migration, invasion and transformation (reviewed in [4]).

Cross-communication between receptor signalling cascades enhances the ability of cells to respond appropriately to different stimuli. In the PC-3 human prostate cancer cell line, LPA stimulates proliferation by activation of ERK1/2 and the tyrosine kinase activity of EGFR. The pathophysiological significance of this mechanism is demonstrated by inhibition of constitutive EGFR activity upon treatment of PC-3 cells with a metalloproteinase inhibitor [19].

Although abundant evidence has been put forward suggesting that the mitogenic effect of LPA is mediated through LPA\(\_1\), LPA\(\_2\) [11,24] or LPA\(\_3\) [25], using degradation-resistant phosphate analogues of LPA, stereoselective agonists of the LPA receptors, and LPA receptor null cells, Lynch and co-workers demonstrated that the mitogenic effects of LPA are independent of LPA\(\_1\)–LPA\(\_3\) [26]. Thus, similar to many reports showing that the mitogenic effect of S1P might be independent of its receptors [27,28], LPA may also have direct intracellular actions. In this regard, a novel intracellular action of LPA has been uncovered. Some evidence suggests that membrane fission, which occurs whenever a vesicle is produced, is controlled by endophilin I, a cytosolic protein that converts LPA into PA (phosphatidic acid) by the addition of the unsaturated fatty acid, arachidonate [29]. This results in negative membrane curvature by converting an inverted-cone-shaped lipid (LPA) into a cone-shaped lipid (PA) in the cytoplasmic leaflet of the bilayer, mediating synaptic vesicle invagination and fission [29].

**LPA metabolism: intracellular production and extracellular release**

Because LPA is present at high levels in human ascites fluid [30], it is considered to be a mediator as well as an indicator of ovarian cancer [11,12]. LPA has long been known to be produced in cells as an intermediate in lipid synthesis. While bioactive LPA can also be produced from PA by phospholipases in ovarian and prostate cancer cells [31,32], an important recent discovery relevant to cancer was that autotaxin, a secreted protein known to be involved in tumour invasion, vascularization and metastasis, is a lysophospholipase D that converts extracellular lysophosphatidylcholine into LPA [33]. This finding adds support to the notion that LPA plays an important role in tumorigenesis.

Yet another potential pathway for synthesis of LPA is the phosphorylation of monooacylglycerols by a specific lipid kinase [34], an enzyme that has remained an enigma for more than 40 years. We have now cloned and characterized a new lipid kinase which we called AGK (acylglycerol kinase) since it catalyses the phosphorylation of both monooacylglycerol to form LPA and diacylglycerol to produce PA, another potent lipid second messenger that mediates mitogenic activation of mTOR (mammalian target of rapamycin) signalling [35].

**AGK catalyses the phosphorylation of acylglycerols to generate LPA and PA**

Because sequence comparisons revealed that AGK is related to the diaclyglycerol kinase family and the sphingosine kinase family, it was necessary to establish that it was a bona fide AGK. Importantly, when AGK, but not a kinase dead mutant of AGK, was overexpressed, intracellular levels of LPA and PA were increased, without detectable effects on levels of ceramide, sphingosine, or S1P [36]. In agreement, monooacylglycerols and diacylglycerols are substrates for recombinant AGK, while neither sphingosine nor ceramide...
were phosphorylated. Furthermore, when AGK expression was down-regulated by transfection with siRNA (small interfering RNA) targeted to AGK, LPA and PA levels were decreased. Interestingly, AGK has a similar substrate preference profile as a previously described crude bovine monacylglycerol kinase activity [37,38], showing higher activity with acylglycerols containing a C₁₈ fatty acid with a single double bond, although arachidonoylglycerol, an endogenous cannabinoid containing a C₂₄ fatty acid with four double bonds, was a reasonably good substrate.

**AGK: a key player in survival signals induced by EGF**

Proliferation of many types of cancer cells is controlled in part by an autocrine stimulatory loop, since EGFR is often overexpressed in transformed cells that also produce ligands that can transactivate the EGFR by activating their own receptors. Several lines of evidence suggest that AGK, which is highly expressed in prostate cancers, could play a role in prostate cancer progression. Overexpression of AGK in prostate cancer cells increased the formation and secretion of LPA, resulting in transactivation of EGFR and activation of the downstream MAPK (mitogen-activated protein kinase) signalling pathway leading to increased cell growth. Importantly, when endogenous AGK expression was ablated in these prostate cancer cells, EGF-induced ERK1/2 activation and cell proliferation were markedly inhibited [36]. Moreover, down-regulation of AGK decreased EGF-mediated cell motility, which plays an important role in androgen-refractory prostate cancer.

The endocannabinoids anandamide and 2-arachidonoylglycerol are known to induce apoptosis of PC-3 and DU145 prostate cancer cells [39]. As AGK can phosphorylate 2-arachidonoylglycerol, converting it into LPA, it can influence the dynamic levels of these counterregulatory lipids that have opposing effects on growth and survival of prostate cancers. Mono-oleoylglycerol, the best substrate for AGK, is phosphorylated to form C₁₈:₁ LPA, and LPA species with unsaturated fatty acids, in particular, C₁₈:₁ and C₁₈:₂ LPA, are much more potent than their saturated counterparts in stimulating the growth of ovarian [40] and prostate cancer cells [32]. In this regard, it has been suggested that increased IP₃ levels with unsaturated fatty acid chains may be associated with late-stage or recurrent ovarian cancer [41]. LPA with unsaturated fatty acids preferentially stimulates LPA₃, whereas LPA₁ and LPA₂ receptors show broader ligand specificities [42]. Prostate cancer cells express LPA₁–LPA₃ receptors and thus AGK can potentially regulate numerous growth signalling pathways downstream of these receptors. Although the mitogenic effects of LPA can be LPA₁–LPA₃ dependent [26], in prostate cancer cells, LPA transduces G-protein-dependent mitogenic signals [7,43]. In agreement, our recent results suggest that the growth promoting effects of AGK are mediated via LPA receptors [36]. First, in cells lacking expression of any of the LPA receptors, AGK had no effect on DNA synthesis. Secondly, PTX pretreatment decreased the growth promoting effects of AGK. In agreement, PTX inhibits proliferation of PC-3 cells induced by LPA and serum [7]. Thirdly, a selective antagonist of PPAR-γ inhibited proliferation of vector transfected PC-3 cells, yet it did not abrogate the mitogenic effect of AGK.

Down-regulation of AGK reduced ERK1/2 activation induced by EGF, but not by LPA, the AGK product, suggesting that AGK plays an important role in EGF-induced mitogenic ERK signalling. Nonetheless, AGK also phosphorylates diacylglycerol to produce the bioactive mediator PA, which regulates numerous biological processes including Raf translocation to the plasma membrane [44], activation of mTOR [35,45], vesicle transport [46] and cytoskeletal structure [47]. A recent study demonstrated that PA produced at the ER (endoplasmic reticulum) in yeast is not only an essential ubiquitous metabolic intermediate but also an important signalling lipid [48]. PA on the ER is directly bound to the soluble transcriptional repressor Opil1p to maintain it in an inactive state outside the nucleus. Metabolism of PA releases Opil1p and allows its nuclear translocation and repression of target genes that regulate phospholipid biosynthesis. Our results also imply that specific pools of PA may play important roles in signalling in mammalian cells.

At first glance, localization of AGK in the mitochondria seemed odd. However, it should be noted that an LPA phosphatase with 28.5% amino acid identity to human prostatic acid phosphatase is also localized to the mitochondria [49], and regulates lipid metabolism in the mitochondria by degrading LPA to monoacylglycerol [49]. More than a decade ago, it was suggested that LPA produced in the mitochondria can be transported to the ER in the presence of liver fatty acid binding protein, be secreted and/or converted into PA.
[49–52]. Moreover, prostate cancer cells can secrete LPA generated by mitogenic stimuli [6–8,32,53,54]. Because these cells also express LPA1–LPA3, it has been suggested that LPA can act as an autocrine mediator [32]. However, the enzymes that produce LPA in prostate cancer cells have not been conclusively identified. Our results suggest that production of LPA by AGK, which in turn transactivates the EGFR, can amplify mitogenic and survival signals. In addition, EGF, serum and LPA itself increase the expression of AGK, thus acting in a positive feed-forward manner that could enhance EGFR-dependent and -independent processes important for cancer progression (Figure 1). Hence, targeting AGK could offer additional therapeutic benefits in treatment of androgen-independent prostate cancer.

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