Targeting a Bioactive lipid in PI3K-induced Oncogenicity

Student: Patricia Jean Eror Barnes ● Supervisor: George Poulogiannis

Patricia Jean Eror Barnes and Dr. George Poulogiannis

Introduction and Aims

LPA, a mitogenic lipid species overproduced in cancers, is important for wound healing and chemoattraction and has been associated with all ten hallmarks of cancer[1,2]. LPA has been claimed to activate oncogenic PI3K signalling, however due to its complex synthesis and metabolism, it has been difficult to target therapeutically (Fig. 1). Mutations in the PI3K pathway are found in over 35% of cancer cases and mainly target the p110α isoform of PI3K, PTEN or AKT [1]. While it has been an appealing therapeutic target, pharmacological inhibition of this pathway has been constrained due to acquired drug resistance and toxicity[3]. In certain cases, synergistic treatments with diet and drugs can be potentially more effective than drugs alone [4]. The aim of this project was to investigate the relationship between these two pathways and capture metabolic vulnerabilities that could be utilised in a therapeutic setting to combat PI3K pathway mutant cell lines.

Material and Methods

Cell culture: MCF-10A breast epithelial cells with either wild-type (WT) or mutant (MUT) *PIK3CA* (E545K or H1047R) and/or WT or MUT AKT (E17K) were maintained in Dulbecco's Modified Eagle's Medium/F12 prepared following previously established protocols[5]. 24-hour post-seeding, media was changed to Fatty Acid-Free (FAF) media.

ELISA: Following seeding of MCF-10A cells in culture medium for 24 hours, the cell monolayer was rinsed with PBS and the media was replaced with fatty acid free (FAF) media containing ω - 6:3 fatty acids (FAs) in ratios of 1:1, 1:2, and 10:1. using docosahexaenoic acid (Sigma #53171), eicosapentoic acid (Sigma #E2011) and arachidonic acid (Sigma #10931) Quantification of LPA was carried out 24 hours post-media change with an ELISA Kit (Cloud Clone Corp, CEK623Ge) according to the manufacturer's protocol. Statistical analysis was performed using two-way ANOVA in GraphPad and Excel.

Spheroids: 1000 cells were seeded in low-adherence 96-well plates and centrifuged for 5 min at 1,200 rpm. Spheroids formed at 37°C over a period of 3 days. On day 3, Matrigel (Corning, 354230) containing 23μM LPA (Sigma, L7260) was placed on top of the spheroid. Photographs were taken every 24 hours. ImageJ software was used to quantify invasion. Data were analysed in Excel.

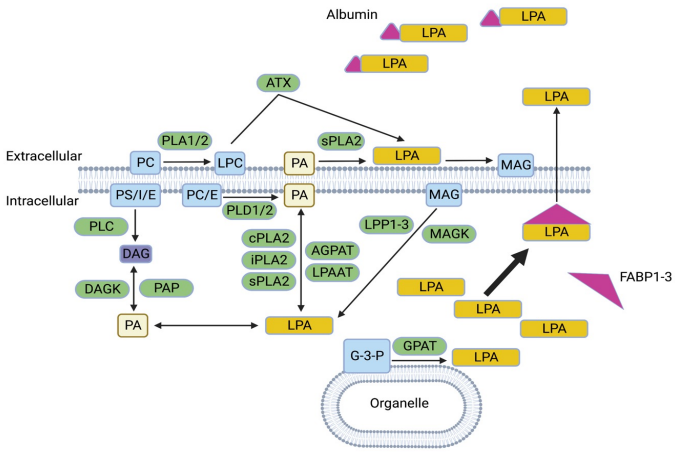


Figure 1. Overview of LPA synthesis. PC: phosphatidylcholine, LPC: lysophosphatidylcholine, MAG: monoacylglycerol, PS: phosphatidylserine, PI: phosphatidylinositol, PE: phosphatidylethanolamine, G3P: glycerol-3-phosphate, PA: phosphatidic acid, DAG: diacylglycerol. Enzymes include ATX: autotaxin, PLA1/2: phospholipase A1 and A2, sPLA2: secretory phospholipase A2, PLC: phospholipase C, DAGK: diacylglycerol kinase, PAP: phosphatidic acid phosphatase, cPLA2: cytosolic phospholipase A2, iPLA2: independent phospholipase A2, AGPAT: acyl-glycerol-3-phosphate acetyltransferase, LPAAT: 1-acyl-glycerol-3-phosphate acetyltransferase, LPP1-3: lipid phosphate phosphatase, MAGK: monoacylglycerol kinase, GPAT: glycerol-3-phosphate acetyltransferase. Image generated in BioRender.

Results

A 2:1 ratio of ω-3 to ω-6 fatty acids reduces LPA levels in *PIK3CA* mutant cells. PLA_2 enzymes in the LPA synthesis pathway show phospholipid substrate specificity-dependent composition of the sn-2 position of the phospholipid[6]. To measure LPA levels in response to fatty acid availability, *PIK3CA* isogenic mutant cells were grown in FAF media containing ratios of 1:1, 1:2 and 10:1 ω-6 to ω-3 fatty acids. Interestingly, *PIK3CA* mutant cells exposed to media with a ratio of 1:2 ω-6 to ω-3 FAs showed a reduction in extracellular LPA levels equivalent to the levels observed in WT cells (Fig. 2).

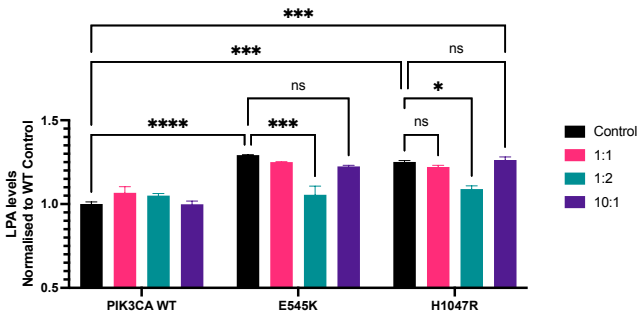
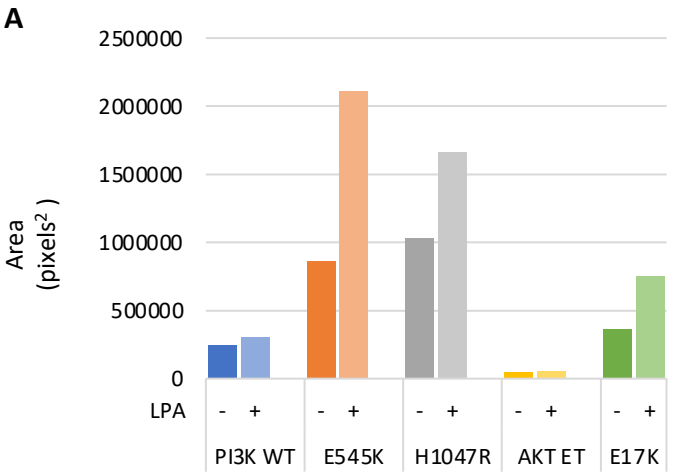


Figure 2. 1:2 ratio of omega-3 to omega-6 shows a reduction of LPA levels. Extracellular levels of LPA in MCF-10 mutagenic cell lines * = P < 0.05, *** = P≤ 0.001, **** = P ≤ 0.0001 The data above was analysed using two-way ANOVA and multiple comparisons test.

LPA enhances invasion To investigate the effect of LPA on cell invasion, matrigel-embedded spheroids of *PIK3CA* isogenic MUT cells were treated with LPA. Interestingly, LPA treatment enhanced the invaded surface area of the cells, with the difference being more prominent in *PIK3CA* H1047R- and AKT E17K- MUT cells (Fig. 3A-B).



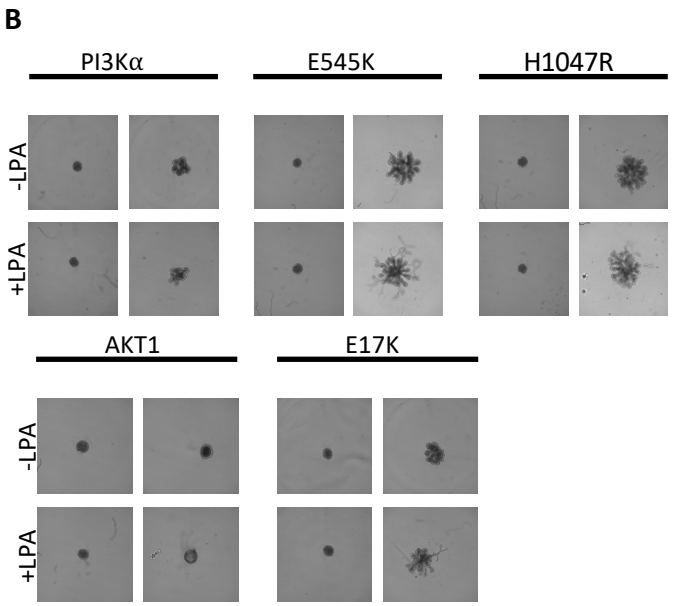


Figure 3. LPA enhances matrigel invasion of MCF-10A isogenic cells. (A) MCF-10A *PIK3CA WT*, E545K H1047R, AKT WT and E17K spheroid growth 5 days after LPA treatment. (B) Representative images of spheroids at day 0 and day 5.

Discussion

In this study, we showed that elevated levels of LPA observed in the context of hyperactivated PI3K/AKT signalling can be rescued when cells are cultured in media with a 1:2 ω-6:3 FA ratio. The tested ratios align with profiles of modern diets representing balanced (1:1), healthy (1:2) and a conservative representation of the current Western (10:1) diet[7]. This suggests that LPA synthesis in *PIK3CA* MUT cells has a dependency on the profile of FAs available to the cell (Fig. 2) .

To investigate if elevated LPA influenced cells phenotypically in the context of PI3K/AKT signalling, we performed an invasion assay. All cell lines tested exhibited greater invasion capacity into the matrigel following LPA treatment with a more prominent increase observed in *PIK3CA* MUT lines (Fig. 3).

This experiment falls in agreement with the established role of LPA as a potent oncometabolite that induces tumour cell aggressiveness. Further experiments are required to verify the functional significance of our findings *in vivo*.

Overall, it has been shown that LPA levels in *PIK3CA* MUT cells are influenced by the omega 6:3 FA ratio, and increased LPA abundance promotes oncogenic invasion. Targeting oncogene-induced overgrowth is challenging, but it is tempting to speculate that pharmacological inhibition of the PI3K/AKT pathway with simultaneous deprivation of LPA precursors could be the key to improving therapeutic outcomes in PI3K/AKT-mutant cancers.

Departure from original Proposal

Phosphatidylcholine, a precursor of LPA, was originally to be tested in the context of *PIK3CA* mutation. Instead, experiments focused on the role of omega 6:3 fats to align with ongoing work within the lab.

Future Directions

Further investigation of LPA synthesis in response to changes in signalling and diet will continue to determine the mechanistic relationship between oncogenic PI3K/AKT signalling and LPA metabolism and help to identify novel metabolic vulnerabilities for PI3K/AKT-mutant cancers.

Value of Studentship

To the student: I am grateful for this studentship for the invaluable opportunity it gave me to work in the highly collaborative Poulogiannis' Signalling and Cancer Metabolism lab at the ICR. It was an honour to work in this world-class facility with experts in their field. I look forward to using the biochemical and molecular techniques I learned here in my future research career.

To the lab: Patricia has been an exceptional student and it was great to see her enthusiasm and drive for research. The data that she generated will undoubtedly help with further elucidating the functional significance of PI3K pathway-induced metabolic reprogramming.

References:

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