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Cancer Cells Incorporate and Remodel Exogenous Palmitate into Structural and Oncogenic Signaling Lipids

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Abstract

De novo lipogenesis is considered the primary source of fatty acids for lipid synthesis in cancer cells, even in the presence of exogenous fatty acids. Here, we have used an isotopic fatty acid labeling strategy coupled with metabolomic profiling platforms to comprehensively map palmitic acid incorporation into complex lipids in cancer cells. We show that cancer cells and tumors robustly incorporate and remodel exogenous palmitate into structural and oncogenic glycerophospholipids, sphingolipids, and ether lipids. We also find that fatty acid incorporation into oxidative pathways is reduced in aggressive human cancer cells, and instead shunted into pathways for generating structural and signaling lipids. Our results demonstrate that cancer cells do not solely rely on *de novo* lipogenesis, but also utilize exogenous fatty acids for generating lipids required for proliferation and protumorigenic lipid signaling.

Keywords

Cancer metabolism; lipid signaling; metabolomics; lysophosphatidic acid; ceramide-1-phosphate; platelet activating factor

1. Introduction

Heightened *de novo* lipogenesis is a fundamental hallmark of nearly all cancers and is required for cellular transformation and cancer progression¹. Fatty acid synthase, the enzyme responsible for *de novo* synthesis of fatty acids, is upregulated across multiple types of human tumors and blocking FASN has been shown to attenuate cell proliferation, tumorigenicity, and cancer malignancy¹. Early studies, using radioactivity-based methods measuring bulk lipids, have shown that *de novo* synthesis of fatty acids from glucose and other carbon sources account for 93 % of the total cellular lipid content in certain cancer types². Cancer cells are thus thought to rely almost solely on *de novo* lipogenesis, rather

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than exogenous fatty acids for generation of cellular lipids³. In addition to lipogenic pathways that subserve cancer proliferation, we have previously shown that aggressive human cancer cells also upregulate lipolytic pathways to mobilize free fatty acids to generate oncogenic signaling lipids that in-turn fuel aggressive features of cancer⁴. We found that the tumorigenic impairments conferred by inactivating a lipolytic enzyme monoacylglycerol lipase (MAGL) in cancer cells, could be rescued by exogenous fatty acids *in situ* or by high-fat diet feeding *in vivo*⁴. These results put forth the possibility that exogenous fatty acids, despite the dominant role of *de novo* fatty acid synthesis, may also play an important role in cancer pathogenesis.

In this study, we investigated whether cancer cells are capable of incorporating exogenous free fatty acids (FFA) and used advanced metabolomic platforms to comprehensively understand how FFAs are remodeled within cancer cells, and whether this exogenous FFA-derived lipid metabolism is altered during cancer progression.

2. Materials and Methods

2.1 Cell Culture

C8161, MUM2C, 231MFP, MCF7, SKOV3, OVCAR3, PC3, and LNCaP cells were obtained from Benjamin Cravatt at The Scripps Research Institute or from ATCC. MCF10A, M2, M2T, and M4 cells were obtained from Stefano Piccolo at the University of Padua⁵. Cells were cultured as previously described^{4,6}.

2.2 Isotopic fatty acid labeling of cancer cells and mice

Cancer cells were seeded (1.5×10^6 cells) and upon adherence, cells were serum starved and treated with d₀-palmitic acid or (7,7,8,8-d₄)-palmitic acid (10 μ M in 0.5 % BSA) for 4 h. Cells were then washed twice in phosphate-buffered saline (PBS) and harvested by scraping. Cells were collected on ice and centrifuged at $1000 \times g$ and cell pellets were frozen at -80°C until lipid extraction.

For isotopic fatty acid labeling of mouse tumor xenografts *in vivo*, M4 cancer cells (2×10^6 cells) were subcutaneously injected into the flank of immune-deficient SCID mice and tumors were grown out to 800 mm³. Mice were treated with vehicle or d₄-palmitic acid (100 mg/kg oral gavage in polyethylene glycol 300 (PEG300)) for 4 h. Mice were then sacrificed and tumors were removed and flash frozen.

2.3 Metabolomic analyses

Cells and tumors lipids were extracted as previously described⁴. Briefly, cells and tumors were extracted in 2:1:1 chloroform:methanol:phosphate-buffered saline with inclusion of internal standards (10 nmoles of dodecylglycerol and 10 nmoles of pentadecanoic acid). The organic layer was collected and the remaining aqueous layer was acidified with 0.1 % formic acid and re-extracted in chloroform. Organic layers were combined and dried down under a stream of nitrogen. Dried extracts were resolubilized in 120 μ l of chloroform and an aliquot (10 μ l) was injected onto an Agilent 6430 triple quadrupole (QQQ)-liquid chromatography-mass spectrometry (LC-MS) instrument.

Targeted mass spectrometry analysis was performed as previously described⁷. Briefly, single-reaction monitoring (SRM) programs were derived from nonisotopic standards and databases. SRM programs for isotopic lipids were based on the ms² fragments and optimized collision energies of nonisotopic standards. Metabolites were quantified by integrating the area under the curve and normalized against internal standards and external standard curves.

Untargeted mass spectrometry analysis was performed by LC-MS in scanning mode collecting mass spectral data from m/z 50-1200. Data files were extracted as mzdata files and analyzed by XCMSOnline (xcmsserver.nutr.berkeley.edu) to identify isotopic fatty acid incorporation into cellular lipids^{4, 8}. Structures of metabolites from untargeted analysis were identified based on database searches (METLIN⁹) and incorporation of d_4 -palmitate, as well as co-elution of metabolites with standards within the same class of metabolites.

Lipids were separated by reverse phase chromatography with a Luna C5 column (Phenomenex) starting with 100 % 95:5 water:methanol with a gradient to 100 % 60:35:5 isopropanol:methanol:water as previously described. Formic acid (0.1 %) with 50 mM ammonium formate or ammonium hydroxide (0.1 %) was added for positive and negative ionization mode, respectively. Metabolites were quantified by integrating the area under the curve, normalizing to internal standards, and then calculating levels based on external standard curves with representative lipid standards from each lipid species. For those metabolites for which there was a background peak for the isotopic d_4 -lipid in the d_0 -C16:0 FFA-treated group, we subtracted the average of the background from both d_0 - and d_4 -C16:0 FFA-treated groups. For all metabolites, the isotopic d_4 -lipid peak for the d_0 -C16:0 FFA-treated group was less than 20 % of the d_4 -C16:0 FFA-treated group.

3. Results

3.1 Isotopic Fatty Acid Tracing-Based Metabolomics Reveals that Cancer Cells Incorporate Exogenous Fatty Acids into Structural and Signaling Lipids

To understand how cancer cells incorporate exogenous lipids and whether this lipid metabolism is altered during cancer progression, we treated a panel of aggressive versus non-aggressive human cancer cells from breast, ovarian, prostate, and melanoma cancers *in situ* with nonisotopic or isotopic palmitic acid (C16:0 free fatty acid (C16:0 FFA)), 10 μ M in 0.5 % fatty-acid free BSA for 4h). These aggressive human cancer cells (231MFP, SKOV3, PC3, and C8161) have been previously shown to possess heightened motility, invasiveness, and *in vivo* tumor growth rates, compared with their non-aggressive counterparts (MCF7, OVCAR3, LNCaP, and MUM2C)^{4, 6}. We also profiled a human breast cancer progression model consisting of: 1) MCF10A nontransformed mammary epithelial cells; 2) MCF10A cells transformed with the activated HRAS (MCF10A-T1k cells or M2 cells); 3) M2 cells transduced with the constitutively activated transcription factor TAZ S89A (M2T cells) that have been previously shown to induce epithelial-to-mesenchymal transition (EMT), poor breast cancer prognosis, and stem-cell-like features in breast cancer; and 4) M4 (or MCF10A-CA1a) cells that are malignant derivatives of M2 cells through spontaneous malignant evolution *in vivo*. These cells are highly tumorigenic, metastatic, and display increased stem-like features and an upregulation of TAZ⁵. We then extracted the lipidome of these cells and quantitatively measured isotopic incorporation into cellular lipids using a combination of targeted SRM-based and untargeted discovery-based metabolomic profiling¹⁰ to globally track the isotopic incorporation and remodeling of exogenous fatty acids into cancer cells (**Fig. 1**). Our SRM methods included ~60 representative lipid species that could potentially incorporate isotopic palmitate, including phospholipids, neutral lipids, sphingolipids, and ether lipids. Our untargeted methods collected mass spectrometry data over a large mass range (m/z 50-1200) and subsequent datasets were analyzed by the XCMSOnline software⁸ to integrate all detectable ions (~5000-10,000 ions), and identify significant alterations in the metabolomes. We combined both targeted and untargeted data to gain a global understanding of exogenous FFA-derived lipid metabolism in cancer cells and mapped these data onto metabolic pathway maps.

Our metabolomic profiling of isotopic palmitic acid incorporation revealed that cancer cells robustly incorporate exogenous fatty acids into cancer cells, which are in-turn remodeled

into acyl carnitines (AC), phospholipids such as phosphatidyl cholines (PC), lysophosphatidyl cholines (LPC), phosphatidic acids (PA), lysophosphatidic acids (LPA), phosphatidyl ethanolamines (PE), lysophosphatidyl ethanolamines (LPE), phosphatidyl inositols (PI), phosphatidyl glycerols (PG) and phosphatidyl serines (PS), neutral lipids such as triacylglycerols (TAG) and diacylglycerols (DAGs), sphingolipids such as ceramide, sphingomyelin (SM), and ceramide-1-phosphate (C1P), and ether lipids such as alkyl PCs, alkyl PEs, alkyl PIs, alkyl PGs, alkyl PSs, alkyl PIs, platelet activating factor (PAF), and lysoPAF (**Fig. 2A-E; Supplemental Table 1**). These incorporated lipids not only include structural lipids (e.g. PC, LPC, SM, PE, LPE, PS, PG, PI, alkyl PCs, alkyl PEs, alkyl PIs, alkyl PGs, alkyl PSs, and alkyl PIs) and lipid stores (e.g. TAGs and DAGs), but also signaling lipids such as LPA, ceramide, DAG, and C1P. Several of these signaling lipids, such as LPA, DAG, and C1P or their associated signaling pathways have been shown to promote cancer pathogenicity¹¹. We also find that C16:0 FFA also contributes to the generation of C18:0 FFA (stearic acid) and is incorporated into several C18:0 FFA-containing lipids. As such, with our targeted methods monitoring the m/z 184 phosphocholine ms2 fragment, we acknowledge the possibility that C16:0 PAF (1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) may be a combination of C16:0 PAF and C18:0 LPC (1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine).

3.2. Isotopic Fatty Acids are Incorporated into Oncogenic Signaling Lipids in Tumors *in Vivo*

We also wanted to investigate whether fatty acids can be incorporated *in vivo* into tumor xenografts in mice. Mice bearing M4 tumors were treated with d₄-C16:0 FFA (100 mg/kg oral gavage, 4 h), and isotopic incorporation into tumor lipids was measured by mass spectrometry. Consistent with our *in situ* studies, we found that exogenous d₄-C16:0 FFA was incorporated into certain lipid species including LPC, PAF, and C1P (**Fig. 2F**). Our studies suggest that exogenous fatty acid-derived lipids, which include oncogenic signaling lipids PAF and C1P, are found in tumors or tumor-associated cells *in vivo*.

3.2. Fatty Acid Incorporation into Structural and Oncogenic Signaling Lipids are Heightened in Aggressive Cancer Cells

We next wanted to understand alterations in lipid metabolism that may underlie cancer progression. We therefore compared isotopic fatty acid incorporation across aggressive versus non-aggressive cancer cells from multiple cancer types, and filtered for isotopic lipid levels that were commonly altered across three out of the five aggressive cancer cells (231MFP, M4, PC3, SKOV3, and C8161) compared to their non-aggressive counterparts (MCF7, MCF10A, LNCaP, OVCAR3, and MUM2C). We intriguingly found a common signature of altered lipid metabolism shared among aggressive cancer cells in which there are lower levels of isotopically labeled ACs, and increased levels of isotopically labeled phospholipids such as PA, PS, PC, and PI, sphingolipids such as ceramide and SM, ether lipids such as alkyl PE and alkyl PC, as well as oncogenic signaling lipids PAF, LPA, and C1P (**Fig. 2A-E, Fig. 3; Supplemental Table 1**). While we believe that these changes are reflective of reduced or heightened fatty acid incorporation into these lipids, we note that in this comparative analysis, we cannot formally distinguish between alterations in synthetic or degradation rates of each lipid. Using the KEGG Pathway database¹² as a guide, fatty acid incorporation into cellular lipids was mapped onto a pathway diagram. We find that FFA incorporation and remodeling into phospholipid, sphingolipid, and ether lipids is heightened across aggressive cancer cells indicating that aggressive cancer cells rely more heavily on exogenous FFAs for cancer cell lipids (**Fig. 4**).

4. Discussion

Taken together, our results reveal that cancer cells incorporate and utilize exogenous fatty acids not only for generation of cellular membranes for cell division, but also for synthesis of signaling lipids, such as C1P, PAF, DAG, and LPA, that have been previously shown to fuel cancer cell pathogenicity¹¹. While recent studies have shown that carnitine palmitoyltransferase (CPT)1A or CPT1C activity promotes cell survival, tumor growth, or cellular motility in certain types of cancer cells and that blocking CPT may be a novel cancer therapeutic strategy¹³, our data would suggest that CPT and fatty acid oxidation pathways are attenuated during cancer progression to shunt fatty acids from beta-oxidation pathways (i.e. carnitine palmitoyltransferase (CPT)-mediated AC production) to generate more structural and oncogenic lipids. These results are reinforced by our previous genomic profiling efforts showing that the aggressive cancer cells used in this study possess lower levels of CPT expression compared to their non-aggressive counterparts (**Supplemental Figure 1**)⁶. Nonetheless, blocking CPT may be an attractive therapeutic strategy for combatting less aggressive or low-grade tumors.

Of particular interest are the exogenous fatty acids that are incorporated significantly more into the signaling lipids C1P, PAF, and LPA across several types of aggressive human cancer cells compared with their less aggressive counterparts. C1P is formed by ceramide kinase and has been shown to oppose the apoptotic effects of ceramide and promote cell proliferation and survival through activating intracellular signaling pathways such as MEK, ERK, PI3K/AKT, and JNK, activate inflammatory responses by activating cytosolic phospholipase A2 for generating pro-inflammatory prostaglandins, and stimulate cell migration through stimulating a yet unknown extracellular G_i-coupled receptor^{11c}. PAF, an inflammatory lipid that acts through PAF receptors and causes inflammation and platelet aggregation, has also been shown to be produced by melanoma cancer cells and promote invasiveness and metastasis through stimulating cancer cell PAF receptors in an autocrine mechanism¹⁴. LPA is a potent oncogenic signaling lipid that acts through stimulating LPA receptors leading to activation of multiple downstream effector pathways including phospholipase C, PI3K-AKT, RAS-ERK, and RHO and RAC GTPases leading to proliferation, survival, migration, invasion, and increased endothelial permeability^{11a}. Increased incorporation of exogenous fatty acids into C1P, PAF, and LPA in aggressive cancer cells can thus potentially fuel cancer initiation, progression, and metastasis.

Beyond the generation of these oncogenic signaling lipids, we also show that palmitic acid incorporation into complex lipids is globally increased in aggressive cancer cells into glycerophospholipid, sphingolipid, and ether lipid pathways. While there have been many studies into the bioactive roles of glycerophospholipids and sphingolipids^{11b}, the role of ether lipids in cancer remains relatively poorly understood, despite its established correlation with aggressive cancers¹⁵. It will be of future interest to understand the role of heightened ether lipid synthesis in cancer progression.

Previous studies have indirectly suggested that cancer cells utilize exogenous fatty acids for energy or membrane formation. Nieman et al showed that ovarian cancer cells use lipids derived from neighboring adipocyte stores *in vitro* by co-culture of ovarian cancer cells and adipocytes¹⁶. Studies have also shown that adipose stromal cells transplanted into mice promote tumor growth by serving as perivascular adipocyte progenitors. Intratumoral adipocytes can also fuel tumor vascularization and cancer cell proliferation¹⁷.

While we show here that cancer cells take up exogenous free non-esterified palmitic acid, we do not yet understand the mechanism for palmitic acid uptake. Previous studies have shown that breast cancer and sarcoma cells expressing lipoprotein lipase and CD36,

involved in lipoprotein-associated triglyceride lipolysis and fatty acid transport, respectively, treated with triglyceride-rich lipoproteins led to accelerated cell proliferation¹⁸. These authors also found that providing lipoprotein lipase to prostate cancer cells with triglyceride-rich lipoproteins prevented the cytotoxic effects of fatty acid synthesis inhibition. The expression of fatty acid binding proteins that are involved in fatty acid uptake and transport have also been associated with poor survival in triple-negative breast cancers¹⁹. Intriguingly, Kamphorst et al recently demonstrated that under hypoxic conditions or Ras activation, cells switch from *de novo* lipogenic pathways to scavenging of serum fatty acids esterified to lysophospholipids to fuel membrane production²⁰. Interestingly, this study also shows that this phenomenon is also linked to reduced glycolytic flux to acetyl-CoA and an increased flux of glutamine to fatty acid synthesis. While these authors were also unable to ascertain the mechanism of lysophospholipid import, they show yet another mechanism through which cancer cells take up fatty acids sources. It will also be of future interest to determine the interplay between glycolytic and glutamine metabolism and fatty acid uptake and metabolism during cancer progression.

Our results provide a potential alternate and more direct mechanism linking obesity to increased incidence of cancer deaths by directly taking in exogenous fatty acids into structural and signaling lipids that can drive cancer pathogenicity. This mechanism adds to previous studies linking obesity-induced inflammation, hyperinsulinemia and increased insulin growth factor signaling, and heightened adipokine signaling to cancer cell proliferation and malignancy²¹.

In summary, we have used advanced metabolomic platforms to globally map exogenous fatty acid incorporation and metabolism into cancer cells *in situ* and *in vivo*. We find a commonly dysregulated metabolic signature of lipid metabolism that underlies aggressive human cancer cells where there is an overall increase in exogenous fatty acid incorporation that is redirected from oxidative pathways to the generation of structural and signaling glycerophospholipids, sphingolipids, and ether lipids. Targeting fatty acid uptake into cancer cells, in combination with inhibitors of key nodal lipid metabolism pathways may provide a potential alternate strategy for treating cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

FFA	Free fatty acid
QQQ-LC/MS	triple quadrupole liquid chromatography-mass spectrometry
MAGL	monoacylglycerol lipase
PEG	polyethylene glycol
EMT	epithelial-to-mesenchymal transition
SRM	single reaction monitoring

AC	acyl carnitines
PC	phospholipids such as phosphatidyl cholines
LPC	lysophosphatidyl cholines
PA	phosphatidic acids
LPA	lysophosphatidic acids
PE	phosphatidyl ethanolamines
LPE	lysophosphatidyl ethanolamines
PI	phosphatidyl inositols
PG	phosphatidyl glycerols
PS	phosphatidyl serines
TAG	triacylglycerols
DAGs	diacylglycerols
SM	sphingomyelin
C1P	ceramide-1-phosphate
PAF	platelet activating factor
CPT	carnitine palmitoyl transferase

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Highlights

- Cancer cells robustly incorporate and remodel exogenous fatty acids into structural and oncogenic signaling lipids.
- Aggressive human cancer cells reduce fatty acid incorporation into oxidative pathways, and instead use exogenous fatty acids for generating higher levels of structural and signaling lipids compared to their non-aggressive counterparts

Highlights

- Cancer cells remodel exogenous fatty acids into oncogenic signaling lipids.
- Aggressive cancer cells reduce fatty acid incorporation into oxidative pathways
- Fatty acids are also incorporated into oncogenic lipids *in vivo* in tumor xenografts

Figure 1. Metabolomic mapping of exogenously-derived isotopic FFA metabolism in cancer cells
Cells were treated with either d_0 -C16:0 FFA or d_4 -C16:0 FFA for 4 h. Lipids were extracted and analyzed by LC-MS using targeted SRM-based approaches and untargeted approaches. The large datasets resulting from untargeted metabolomics was analyzed by XCMS Online to determine masses that were altered between d_0 - vs d_4 -C16:0 FFA to identify isotopic-FFA-incorporated lipids.

Figure 2. Mapping exogenous isotopic FFA-derived lipid metabolism in human cancer cells (A-E) Shown on the left are all ions detected in 231MFP, M4, SKOV3, PC3, or C8161 aggressive human cancer cells. For the volcano plot, points that are to the left of the dotted line ($p < 0.05$) represent ions that were not statistically altered in levels between d_0 -C16:0 FFA versus d_4 -C16:0 FFA-labeled cells. All points to the right of the dotted line ($p < 0.05$) represent ions that had significantly higher ion intensity in the d_4 -C16:0 FFA labeled group compared to d_0 -C16:0 FFA labeled group, i.e. d_4 -incorporated lipids. In total, at least ~5,000-10,000 ions were detected and analyzed between targeted and untargeted analysis comparing d_0 -C16:0 FFA labeled to d_4 -C16:0 FFA labeled M4, 231MFP, C8161, SKOV3, or PC3 cells. The y-axis denotes fold-change between raw integrated values of isotopically-

incorporated ions by either targeted or untargeted analysis between d_0 versus d_4 -C16:0 FFA-labeled samples. For the ions that exhibited no background peak corresponding to the m/z of the d_4 -lipid in the d_0 -C16:0 FFA-treated cells, we considered this value to be 1 to obtain a fold-change value compared to the raw integration values of d_4 -C16:0 FFA-treated cells. For the ions for which there was a background peak, we obtained a fold-change value by dividing the ion intensity for the d_4 -C16:0 FFA compared to d_0 -C16:0 FFA groups. The heat-map on the right shows relative levels of d_4 -C16:0 FFA-incorporated lipids in non-aggressive (MCF7, OVCAR3, LNCaP, MUM2C) or non-transformed (MCF10A) cells compared to aggressive (231MFP, M2T, M4, SKOV3, PC3, C8161) or transformed (M2) cells. In the heat-map, relative levels of each d_4 -incorporated lipid metabolite are shown (darker blue shading corresponds to higher level of metabolite). The lipid designations next to the heat map are color-coded red for significantly higher, blue for significantly lower, and grey for unchanged d_4 -metabolites in aggressive cancer cells (231MFP, M4, SKOV3, PC3, and C8161) compared to non-aggressive (MCF7, MCF10A, OVCAR3, LNCaP, and MUM2C, respectively) cells ($p < 0.05$). (F) Shown are lipids species that exhibited significant d_4 -C16:0 FFA incorporation *in vivo* in mice bearing a tumor xenograft from M4 cells. Mice were subcutaneously injected with 2×10^6 M4 cells and tumors were grown out to ~ 800 - 1000 mm^3 . Mice were treated with vehicle (polyethylene glycol 300 (PEG300)) or d_4 -C16:0 FFA (100 mg/kg in PEG) by oral gavage (4 h). Tumors were harvested and lipids were extracted and analyzed by SRM-based metabolomics. For A-F, those metabolites where there was a background peak for the d_4 -lipid m/z in the d_0 -C16:0 FFA-treated cells, the average of the background ion intensity was subtracted from both d_0 and d_4 -C16:0 FFA-treated groups. For all lipid shown here, any background peak for a d_4 -lipid detected in d_0 -C16:0 FFA-treated cells was assumed to either be a coeluting isobaric metabolite or natural isotopic abundance of the lipid. We have only presented here the d_4 -incorporated lipids that showed >5 -fold significantly ($p < 0.05$) higher ion intensity in the d_4 -C16:0 FFA-treated group compared to the d_0 -C16:0 FFA-treated group. All data from A-E is shown in **Supplemental Table 1** and certain lipids are quantified in **Figure 3**. Data in (A-E) are average values of $n=4$ - 6 biological replicates. Data in (F) are mean $\pm$ standard error of $n=4$ - 6 biological replicates. Significance in (F) is represented as $*p < 0.05$ in d_4 -C16:0 FFA-treated mice compared with vehicle-treatment.

Figure 3. Aggressive cancer cells increase incorporation of fatty acids into oncogenic signaling lipids and reduce incorporation into oxidative pathways

Representative lipids with significant fatty acid incorporation from **Figure 2** are quantitated and shown as bar graphs. In comparing d4-C16:0 FFA incorporation into aggressive (C8161, PC3, SKOV3, and 231MFP cells) compared with non-aggressive cancer cells (MUM2C, LNCaP, OVCAR3, and MCF7 cells), we find that there is reduced incorporation into C16:0 AC and increased incorporation into phospholipids, sphingolipids, and ether lipids, including the signaling lipids C1P, PAF/LPC, and LPA. Data are average values of n=4-6 biological replicates and are presented as mean $\pm$ standard error. Significance is represented as *p<0.05 comparing aggressive versus non-aggressive d4-FFA groups.

Figure 4. Map of lipid metabolism in aggressive cancer cells

The data gathered from isotopic tracing of d₄-C16:0 FFA labeled cancer cells was compiled into a metabolic pathway map using the KEGG Pathway Database as a guide. d₄-C16:0 FFA incorporation into the lipid structures is noted in red. The color of arrows and metabolites notes increased (in red), decreased (in blue), or unchanged (in grey) levels of d₄-lipid in three out of five comparisons of aggressive (231MFP, M4, SKOV3, PC3, and C8161) versus non-aggressive (MCF7, MCF10A, OVCAR3, LNCaP, and MUM2C, respectively) cancer cells. Metabolites and arrows in black were not detected in either targeted or untargeted analysis. LPA, C1P, DAG, and PAF are oncogenic signaling lipids that act through LPA receptors, unknown receptor, protein kinase C, and PAF receptors, respectively.