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Imaging of Cancer Lipid Metabolism in Response to Therapy

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Abstract

Lipids represent a diverse array of molecules essential to the cell's structure, defense, energy, and communication. Lipid metabolism can often become dysregulated during tumor development. During cancer therapy, targeted inhibition of cell proliferation can likewise cause widespread and drastic changes in lipid composition. Molecular imaging techniques have been developed to monitor altered lipid profiles as a biomarker for cancer diagnosis and treatment response. For decades, magnetic resonance spectroscopy has been the dominant noninvasive technique for studying lipid metabolite levels. Recent insights into the oncogenic transformations driving changes in lipid metabolism have revealed new mechanisms and signaling molecules that can be exploited using optical imaging, mass spectrometry imaging, and positron emission tomography. These novel imaging modalities have provided researchers a diverse toolbox to examine changes in lipids in response to a wide array of anticancer strategies including chemotherapy, radiation therapy, signal transduction inhibitors, gene therapy, immunotherapy or a combination of these strategies. The understanding of lipid metabolism in response to cancer therapy continues to evolve as each therapeutic method emerges, and this review seeks to summarize the current field and areas of unmet needs.

Abbreviations

- 18F-fluorodeoxyglucose (FDG)
- 2,5-dihydroxybenzoic acid (DHB)
- 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA)
- acetyl-coenzyme A (acetyl-CoA)
- acute myeloid leukemia (AML)
- AMP-activated protein kinase (AMPK)
- ATP-citrate lyase (ACLY)
- beta-oxidation (β -Ox)
- black Hole Quencher (BHQ-3)
- choline kinase (ChoK)
- choline transporters (ChoTs)
- cisplatin (CDDP)
- 7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one (DDAO)
- diacylglycerol (DAG)
- (4',6-diamidino-2-phenylindole) (DAPI_
- elongation-of-very-long-chain-fatty acids (ELOVL)
- endoplasmic reticulum (ER)
- epidermal growth factor receptor (EGFR)
- fatty acid binding protein-4 (FABP-4)
- G-protein coupled receptor (GPCR)
- glycerophosphocholine (GPC)
- hormone-sensitive lipase (HSL)
- hypoxia inducible factor (HIF)
- Indocyanine green (ICG)

1
2
3 isocitrate dehydrogenase-1 (IDH1)
4
5 lipoprotein lipase (LPL)
6
7 low density lipoprotein (LDL)
8
9 lysophosphatidylcholine (LPC)
10
11 magnetic resonance spectroscopy (MRS)
12
13 mass spectrometry imaging (MSI)
14
15 matrix-assisted laser desorption ionization (MALDI)
16
17 mono-unsaturated fatty acids (MUFAs)
18
19 nanostructure-initiator mass spectrometry (NIMS)
20
21 OKN007 (2,4-disulfophenyl-PBN)
22
23 optical coherence tomography (OCT)
24
25 PBN (α -phenyl-tert-butyl nitron)
26
27 peroxisome proliferator-activated receptor alpha (PPAR α)
28
29 phosphatidylcholine (PtdCho)
30
31 phosphatidylethanolamine (PtdEtn)
32
33 phosphatidylinositol (4,5) phosphate-2 (PIP2)
34
35 phosphatidylinositol (PtdIns)
36
37 phosphatidylserine (PtdSer)
38
39 phosphocholine (PC)
40
41 phosphoethanolamine (PE)
42
43 Phosphoinositides (PI)
44
45 phospholipase (PL)
46
47 phosphomonoester (PME)
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49 plasma membrane citrate transporter (PMCT)
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51 polyunsaturated fatty acid (PUFA)
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3 positron emission tomography (PET)
4
5 protein kinase C (PKC)
6
7 PtdCho-specific PLC (PC-PLC)
8
9 pyropheophorbide-a (Pyro)
10
11 reactive oxygen species (ROS)
12
13 secondary ion mass spectrometry (SIMS)
14
15 sphingosine-1-phosphate (S1P)
16
17 stearoyl-CoA desaturase-1 (SCD1)
18
19 sterol response element-binding proteins (SREBPs)
20
21 total choline (tCho)
22
23 tricarboxylic acid (TCA)
24
25 unfolded protein response (UPR)
26
27 α -cyano-4-hydroxycinnamic acid (CHCA)
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Introduction

Cancer cells exist in a hyperactive state of growth and use a complex network of lipid metabolic pathways to support this growing biomass. Lipids are a broad class of compounds that include fatty acids, triglycerides, steroids, phospholipids, and sphingolipids, among others. These molecules play critical roles in cellular compartmentalization, structural barriers, communication signals, energy storage, and homeostasis. Despite high vascularization, many cancer cells exist in regions of nutrient deprivation and tumors have adopted alternative strategies to maintain sources of lipid. In some cases the altered lipid metabolic state offers a therapeutic vulnerability, while in others, adaptations to intracellular lipid composition can be used by refractory tumors to resist therapies. Because these cancer-driven aberrations in lipid metabolism often contrast the surrounding tissue, there are several molecular imaging strategies that have been developed to monitor tumor margin, stage, and treatment response.

I. Lipid Metabolism

A. Exogenous Lipid Uptake

De novo lipid synthesis pathways are crucial during embryogenesis and fetal development, but, after maturation, cells in most tissues are capable of acquiring sufficient circulating lipids to meet their biosynthetic and energetic needs (1-3). Cancer cells can access circulating lipids by overexpressing lipid-scavenging proteins. High exogenous lipids are associated with local invasive index, and obesity is also correlated with higher cancer incidence rates (4). Higher expression of low density lipoprotein (LDL) receptor has been found in transformed colorectal cells relative to normal cells (5). The LDL receptor in prostate cancer provides a major source of cellular cholesterol and essential fatty acids (6). Breast cancer and select sarcoma cells have been observed to secrete lipoprotein lipase (LPL) to release fatty acids

from triglycerides in circulating lipoproteins (7). Although macropinocytosis has been identified in cancer cells, little evidence exists to suggest that this is a relevant source of exogenous lipid *in vivo* (8). Instead, lipid binding proteins are thought to assist in the capture of lipids from the interstitial space and promote invasion (Figure 1), as overexpression of fatty acid binding protein-4 (FABP-4) has been observed on the surface of ovarian, prostate, bladder, and renal cancer cells (9,10). Fatty acid translocase, also known as CD36, is a long-chain fatty acid scavenger that contributes to high mammographic density in subjects at high-risk for breast cancer (11). Ovarian cancer cells can siphon free fatty acids from the lipid stores of the omentum by activating perilipin-A and hormone-sensitive lipase (HSL) in neighboring adipocytes (12). Studies have shown that removal of lipids from culture medium can trigger *de novo* lipid synthesis, emphasizing the dependency on lipids for cell proliferation and the adaptations cancer cells make to sustain their growing biomass (13).

B. De Novo Synthesis

Many cancers revert back to *de novo* lipid synthesis, and the lipogenic pathway is composed of many enzymes that are critical for tumor growth (14). Lipid synthesis starts at the energetic hub of the cell, the mitochondria, where acetyl-coenzyme A (acetyl-CoA) and oxaloacetate are condensed into citrate (Figure 1). Citrate from the TCA cycle can be shunted from the mitochondria and broken down by ATP-citrate lyase (ACLY) into acetyl-CoA. Cytosolic citrate can alternatively be sourced from the microenvironment by plasma membrane citrate transporter (PMCT)(Figure 1) (15). Besides feeding into the rate-limiting step of fatty acid synthesis, acetyl-CoA is also critical for histone acetylation and serves as a link between metabolic status and gene expression (16,17).

Acetyl-CoA serves as the 2-carbon building block used for fatty acid synthesis. The enzyme FASN combines malonyl-CoA with repeated acetyl-CoA condensations to form palmitate. Palmitate is the saturated 16-carbon fatty acid from which most complex fatty acids in the body are derived (18). FASN overexpression is associated with poor prognosis in breast cancer (19) and FASN inhibitors derived from natural products (e.g. resveratrol) and synthetic molecules (e.g. orlistat) are being studied for their anti-neoplastic effects (20). Acetyl-CoA is also a synthetic precursor to 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) in the cholesterol biosynthesis pathway (Figure 1). Mevalonate is then produced by HMG-CoA reductase, the target of the lipid lowering drugs, the statins. The amplification of HMG-CoA reductase in many prostate tumors creates a growth dependency on cholesterol, and suggests a possible role for statins in treating these cancers (5,21). Mevalonate is also a precursor to farnesyl-diphosphate, which can further be processed to cholesterol, or used as a substrate for protein prenylation. Isoprenoids from the mevalonate cascade are critical for membrane anchoring and activation of the growth-related G-protein subunits Ras (farnesylated), Rho (geranylgeranylation), and many others (22) (Figure 1).

Fatty acids can be chemically modified in many ways to meet the diverse range of specialized functions required for cellular function (Figure 1). Stearoyl-CoA desaturase-1 (SCD1) produces mono-unsaturated fatty acids (MUFAs) from the saturated fatty acid chains sourced exogenously or from *de novo* synthesis. Saturated fatty acid accumulation in the endoplasmic reticulum (ER) triggers autophagy-induced apoptosis by activating the unfolded protein response (UPR) (23). By modulating MUFA to saturated fatty acid ratios intracellularly, SCD1 has a regulatory effect on cell survival and proliferation (24-26). The elongation-of-very-long-chain-fatty acids (ELOVL) enzymes in the ER are responsible for polyunsaturated fatty

acid (PUFA) synthesis (27), as well as the conversion of saturated and monounsaturated fatty acids into the very long chain fatty acids ($C > 18$) that serve as building blocks of sphingolipids when combined with ceramide head groups (28). Free fatty acids are stored as triglycerides in lipid droplets or packed into the cell membrane as sphingolipids, cholesterol esters, or phospholipids. Lipid droplets in breast cancer cells have been found to increase with increasing malignancy and to be enriched in polyunsaturated fatty acids, especially arachidonic acid (29)

Diacylglycerol (DAG) is an important lipid second messenger that also serves as a synthetic precursor for both membrane phospholipids and lipid droplet triacylglycerides (30-33). Major membrane phospholipids include phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer), among others, and vary in function, location, and relative abundance. The Kennedy pathway of phospholipid synthesis describes the addition of polar head-groups to the DAG backbone, and enzymes in this pathway are responsible for the accumulation of phosphocholine (PC) and phosphoethanolamine (PE) observed in many cancers (34). Choline uptake by choline transporters (ChoTs) and phosphorylation by choline kinase (ChoK) have, in particular, been consistently linked with increased invasiveness, drug resistance, and overall malignancy (35).

C. Lipid Mobilization and Usage

Phospholipid and triacylglyceride levels are maintained by the concerted actions of catabolic enzymes that mobilize fatty acids from lipid droplets and cell membranes (36). Fatty acid release from lipid droplet storage involves lipases that can support aggressive cancer phenotypes in a manner similar to the pro-tumorigenic effects of exogenous fatty acids (37). Fatty acid release from membrane phospholipids is carried out by the phospholipase (PL) enzymes. PLA₁ and PLA₂ cleave phospholipids at the *sn*-1 and *sn*-2 positions, respectively. The

PLA₂ family of enzymes are the primary producers of arachidonic acid and three major subgroups exist: calcium-dependent or cytosolic cPLA₂, calcium-independent iPLA₂, and secretory sPLA₂ (38). While cPLA₂ has a tumor-supportive role in many cancers, sPLA₂ is thought to have tumor suppressive-functions (39). In contrast, iPLAs are housekeeping enzymes, primarily responsible for mobilizing lipids to maintain membrane integrity and general cellular energy metabolism during homeostasis. PLC cleaves phospholipids at the bond between glycerol and phosphate and contains pleckstrin homology domains for anchoring and cooperation with G-protein coupled receptor (GPCR) signaling networks (40). When activated by growth factor receptors, PLD hydrolyzes the phospholipid head group (e.g. choline, ethanolamine, inositol, serine) to release PA and links growth factor signaling to cell proliferation (32).

Saturated free fatty acids can be broken down into acetyl-CoA to feed into the TCA cycle, and some cancers have been found to prefer fatty acid oxidation to pyruvate oxidation, even in sufficient glucose and oxygen environments (41). β -oxidation is an important energy pathway in prostate cancers, where glycolytic rates are low, and rapid citrate utilization requires a constant supply of acetyl-CoA (42). Peroxisome proliferator-activated receptor alpha (PPAR α) is a transcription factor whose activation triggers breakdown of very long chain fatty acids in the peroxisomes, and fatty acid oxidation in the mitochondria. A complex network of interactions with PUFAs, namely arachidonic and linoleic acids, regulates PPAR α activity (43). Aside from their direct signaling functions (44), arachidonic acid is an important substrate for cyclooxygenase, lipoxygenase, and cytochrome enzymes that catalyze the production of the eicosanoid family of bioactive lipids that have roles in innate immunity, inflammation, cardiovascular disease, and cancer (45,46).

II. Tumor Progression and Regression

A. Oncogenic Reprogramming

A common feature of cancer cells is the metabolic transition from oxidative phosphorylation to glycolysis, during which the rate of glucose consumption far exceeds the energy requirements of the cell (14). What initially appears to be wasteful energy utilization is in fact a method to feed glucose-derived pyruvate into biosynthesis, including fatty acids (Figure 2). Glutamine has been identified in some cancers as an alternative energy source that can enter the TCA cycle (47) and provide a carbon source for citrate production (Figure 2), however the dependency on glutamine for anaplerosis varies considerably even among cancers of the same tissue (48,49). This may be due, in part, to heterogeneity in the local tissue microenvironment causing gradients in nutrients, oxygen and signaling factors that influence the energetic state of cells.

Under hypoxic conditions, solid tumors can become dependent on unsaturated fatty acid uptake from serum to prevent ER stress that can arise when rapid proliferation is unmet by nutrient availability (50). These exogenous fatty acids can arise from lipolysis in stromal cells in a manner comparable to cachexia (23). The recent resurgence of interest in cancer metabolism has improved our understanding of the adaptations tumor cells can make when fuel availability is variable. These adaptations are only possible because the common driving mutations in cancers often have direct involvement in metabolic pathways.

Many of the clinically prevalent oncogenes are capable of altering lipid uptake, production, or consumption to gain a competitive growth advantage (Figure 2). The transcription factor hypoxia inducible factor (HIF) is a downstream effector of Myc and is tightly regulated by the tumor suppressor gene p53. HIF induces FASN induction and lipid droplet formation for

energy storage to support the tumor microenvironment (18,51,52). FASN requires the reducing agent NADPH, which is produced from the pentose phosphate pathway that often becomes upregulated during cancer reprogramming (18). **There is further evidence in renal cell carcinoma models that HIF2 α -dependent lipid storage suppresses the ER stress response promoting tumor cell survival (53).** Glutamine uptake in glioma and acute myeloid leukemia (AML) is often accompanied or driven by isocitrate dehydrogenase-1 (IDH1) mutations that reroute glutamine toward citrate to provide fatty acid and cholesterol precursors independent of the TCA cycle (Figure 2) (54,55). In hypoxic environments where glucose metabolism is diverted to anaerobic lactate production, mutations in mitochondrial IDH2 provide a continued source of citrate for lipid synthesis, by allowing reductive carboxylation of glutamine-derived α -KG (56). The oncometabolite 2-hydroxyglutarate is produced by many cancer-specific IDH1 and IDH2 mutations, and has widespread epigenetic effectors by altering DNA methylation (57-59).

AMP-activated protein kinase (AMPK) serves as an energetic sensor and master switch by inhibiting fatty acid synthesis in low energy states and stimulating consumption of fatty acids for energy in the mitochondria by β -oxidation (Figure 2). Sterol response element-binding proteins (SREBPs) (60) can activate transcription of genes involved in cholesterol and fatty acid synthesis and uptake, and are directly downstream of AMPK and the PI3K/Akt/MTOR signaling axis that includes many oncogenes (61). Epidermal growth factor receptor (EGFR) mutations are common tumor-driving factors that recruit phospholipases (62,63) and ChoK α (64) to the cell surface, leading to membrane remodeling and induction of mitogenic signals to drive cellular proliferation. Mitogenic growth signals can also come in the form of sterol-derived hormones (estrogens, progestogens, and androgens). Release of these sex hormones, their receptor expression, and the downstream signaling mediators are all common tumor-driving factors in

prostate, breast, endometrial and ovarian cancers (65). Farnesylation of Ras and geranylgeranylation of Rho GTPases are essential for downstream signaling through the phosphoinositol family of signaling lipids (66,67). Phosphoinositides (PI) comprise 10-20% of total cell phospholipids and among that fraction, phosphatidylinositol (4,5) phosphate-2 (PIP₂) and phosphatidylinositol-4-phosphate constitute approximately 0.2-1% (68-70). This class of membrane lipids plays an integral role in proliferation, apoptosis, metabolism and migration, and is pivotal to transmembrane signal transduction because it regulates the distribution of receptor tyrosine kinases, G-protein-coupled receptors, and adhesion molecules among others (71).

Metabolic disease is a risk factor for many cancer types, promoting insulin resistance, hyperactive growth signals, and evasion of apoptosis that help set the conditions for malignant transformation (72). Histological tumor grade in the breast is clinically correlated with phosphomonoester (PME) accumulation (73), such as PC and PE, detectable by MR spectroscopy (74,75). PC promotes survival and mitogenic signaling through downstream cascades such as MAPK and Akt (73,76,77). PC can be formed through the actions of the enzyme choline kinase, or by phosphatidylcholine specific phospholipase C. Phospholipid catabolism can also be a source of other mitogenic second messengers such as DAG, PA, and lysophosphatidylcholine (LPC). DAG is released following the cleavage of phospholipids by PLC (78), activates protein kinase C (PKC) resulting in the release of intracellular calcium stores and subsequent Ca²⁺-dependent signaling. DAG and PC are also the synthetic precursors to the most abundant mammalian membrane phospholipid, PtdCho, whose levels are predictive of breast tumor grade, estrogen receptor status, and patient survival (79). It is intuitive to suspect that cancers in high adipose tissue use altered lipid metabolism to take advantage of the surrounding environment, however evidence of altered lipid metabolism in cancers that arise in

low-adipose tissues suggests that deranged lipid metabolism is a universal hallmark of cancer pathogenesis.

Variations in lipid composition are likely due to the heterogeneous nature of the tumor microenvironment, which is an ever-changing compartment of structural fibers, nutrients, cellular waste, and signaling molecules. Interstitial pH, extracellular matrix, bioactive lipids, and cytokines can alter the population and function of stromal cells and surrounding tissues. Extracellular lactate, the metabolic byproduct of aerobic glycolysis, is capable of transitioning tumor-associated macrophages from tumor-suppressive to tumor-supportive phenotypes (80,81). Biopsies of colon cancer specimens have been reported to contain high lipid deposition in regions of necrosis and infiltrating macrophages (82). These macrophages most likely resemble the immune-suppressive “alternatively-activated” phenotype, as classic (interferon gamma-induced) activation does not enhance lipid phagocytosis in human myeloid populations (83). Alternatively-activated macrophages prefer fatty acid oxidation (84) and are thought to have critical roles in clearing/remodeling necrotic tissue, recruiting new blood vessels, and regulating innate and adaptive immunity (85).

B. Lipid changes during Apoptosis/Necrosis

The role lipids play in nearly all cell survival pathways emphasizes their importance in tumor growth and invasion pathways, but lipid compartmentalization, metabolism, and signaling are also intricately involved in cell death pathways. During apoptosis, the release of lysophosphatidylcholine (LPC) and sphingosine-1-phosphate (S1P) by PLAs (86) and ceramidases (87) respectively, acts as a “find-me” signal to surrounding macrophages (88). Once recruited to the dying cell, exposed PtdSer on the apoptotic cell’s surface serves as the “eat me”

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3 signal recognized by phagocytes (89). Unlike the organized compartmentalization of apoptotic
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5 cells into non-immunogenic apoptotic bodies, necrosis is characterized by the breakdown of the
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7 plasma membrane and release of the cellular contents that often triggers an immune reaction
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10 (90). Recently, lipid peroxidase networks have been identified as key mediators of cancer cell
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12 therapy resistance by reversing a non-apoptotic form of cell death known as ferroptosis can be
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14 induced by lipid peroxide accumulation (91). Tumor cell response to therapy begins with
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16 activation of cell stress responses, which can ultimately decide the fate of the cell and
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18 surrounding tumor.
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22 Early changes in lipid metabolism have been reported in response to a wide range of
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24 cellular stresses (92), but lipid mobilization does not necessarily commit a cell to apoptosis (93).
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26 Many cells undergoing apoptosis produce lipid droplets, although there are exceptions (94), and
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28 fatty acids are first mobilized from membrane phospholipids by PLA₂ activity (92,95). Reactive
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30 oxygen species (ROS) produced in the mitochondria of apoptotic cells are thought to inhibit the
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32 catalytically-active thiol groups on β -oxidation enzymes (96), thus free fatty acids are redirected
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34 into lipid droplets in the form of triglycerides and sterol esters (97,98). Lipid droplet formation in
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36 cancer may act as a drug reservoir to reduce intracellular concentrations of drug (99), or to
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38 absorb reactive oxygen species to protect further DNA damage (100). Lipogenesis of saturated
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40 fatty acids also makes the cell membrane less penetrable to drugs that enter by passive diffusion
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42 (101). Uptake of triglycerides by macrophages or neutrophils can dampen subsequent immune
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44 activation (102,103), providing another potential mechanism of immune escape. Ceramide
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46 accumulation regulates and can even trigger mitochondrial outer membrane permeability to fully
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48 commit a cell to apoptosis by allowing cytochrome C release to the cytosol (104). Other signs of
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50 cell stress that precede or arise during apoptosis are the rise in PUFA and glycerophosphocholine
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(GPC) levels (105-107). These indicate a reprogramming of lipid metabolism and suggest that catabolic networks mobilize lipids from membrane phospholipids during this time. Many of these observations were first made using magnetic resonance spectroscopy (MRS) to non-invasively measure lipid resonances of cancer cells undergoing stress responses and apoptosis (92).

III. Imaging Metabolic Lipid Changes

The development of modern imaging techniques allows the detection of several key signaling pathways involved in lipid metabolism and its regulation/dysregulation in cancer. The ability to detect these pathways non-invasively aids in the discovery of potential targets for imaging or interventional therapies. In addition to developing probes to image lipids and alterations in lipid metabolism in mechanisms of oncogenesis, we can also image lipid-dependent or –mediated signaling in response to therapy (108).

A. Imaging lipids in tumors: MR Spectroscopy

MRS has been extensively employed for the study of biological material (from cell extracts, to homogenized tissue, and intact organisms) because it offers a non-invasive method to study the native distribution and dynamic nature of many relevant biomarkers of disease and therapeutic response. The search for disease-related metabolites requires the suppression of the overwhelming fat and water signals observed in proton MR spectra, however much can be learned from the diverse species that contribute to these lipid peaks.

B. Choline and choline metabolites

MRS has been essential in the study of lipid metabolism and for the non-invasive observation of lipid metabolism *in vitro* and *in vivo*. This is due to the ability to observe a number of lipid metabolites in ^1H and ^{31}P MR spectra on the basis of chemical shift discrimination. Due to the relative insensitivity of MR, observation is limited to soluble or MR-visible metabolites in the high micromolar to millimolar levels. Lipid metabolites observable in ^{31}P MR spectra, include the PME, PC and PE and the PDE, GPC and GPE. In ^1H spectra, observable lipid metabolites include the total choline (tCho) resonance, a composite resonance encompassing free choline, PC and GPC. Thus there is overlap in the information available between ^{31}P and ^1H spectra, since the choline PC and GPC resonances seen in the ^{31}P spectra are also observed in the tCho region of ^1H spectra. The PME and PDE resonances report on pathways relevant to phospholipid metabolism. PC and PE are generally the anabolic products of choline kinase and ethanolamine kinases, although they can also be produced by the actions of phospholipase C. The PDE resonances result from catabolism of phosphatidylcholine and phosphatidylethanolamine via the consecutive action of two phospholipases, A2 and lysophospholipase or A1. There are numerous excellent reviews on this topic (35,92,109,110), and the reader is referred to these for detailed information. However, it is worth noting that consistent elevations in **PMEs** and tCho have been observed in a wide range of tumors including brain, prostate, lung, skin, ovarian and breast (111,112). As a means to monitor therapeutic response, tumor choline levels have been measured using MRS in animal (113-115) and human tumors (116-120) with mixed results, at least partly due to the technical difficulties associated with acquiring choline spectra on a background of high fat in normal breast tissue. The picture is further complicated because ChoK is not the only enzyme that contributes to PC accumulation, it

can be produced directly by the actions of PLC on PtdCho, or by the hydrolysis of GPC as a source of additional choline for subsequent phosphorylation. Recent MRS applications have been used to detect changes in choline metabolism due to IDH mutations in glioma (59,121) explore the role of the glycerophosphodiesterase genes GDPD5 and GDPD6 on breast cancer cell migration/invasion (122) and profile metabolic changes in response to HIF1 and HIF2 suppression (123).

C: Imaging lipids in tumors: MR-visible lipids

Proton MRS was originally used to detect MR-visible or mobile lipid signals in cultured cells and tumor biopsies. A series of resonances were observed arising from the fatty acryl chains in neutral lipids, triglyceride and cholesterol esters, including the terminal methyl groups, the methylenes in long chain fatty acids, and olefinic MUFA/PUFA resonances (124-127). MR detects only molecules that exist in a local environment with high rotational molecular motion and thus the observed signals arise predominantly from mobile or MR-visible lipids, composed of triglycerides and cholesterol esters sequestered in lipid droplets. In animal and human tumors, the observation of these lipid resonances require the use of short echo time spectroscopic pulse sequences due to the relatively short T_2 relaxation times of mobile lipids. *In vivo* MRS studies initially used single voxel localization methods such as STEAM and PRESS for assessing the MRS pattern of lipids in tumors, due to the simplicity and accuracy of these methods. However, multi-voxel spectroscopic methods including chemical shift imaging are increasingly being used both in mouse glioma and xenograft models (128,129) as well as in human tumors (130) since these methods provide a better assessment of tumor heterogeneity. The presence of intense lipid signals from surrounding subcutaneous fat and muscle have been problematic in evaluating lipid signals by MRS methods, thus outer volume fat suppression sequences become critical. For this

reason, the majority of studies on intra-tumoral lipids have been focused on the brain where subcutaneous fat is at a minimum. However, useful insights into human tumor composition have still been made in other tumors, despite the problems associated with high fat content in peripheral tissue.

The presence of lipids in human tumors was initially reported by Kuesel *et al.* (124) where a correlation between MR-visible lipids, tumor malignancy and necrosis was observed in brain tumors. These initial findings were subsequently confirmed in *in vivo* imaging studies as well as in histologically different brain tumor types (131). An early demonstration of the sensitivity of lipid signals for accurate diagnosis of supra-tentorial brain tumors was demonstrated by Preul *et al.* (132) suggesting lipid signals as a potential biomarker for grading of astrocytomas. Correlation of lipids with malignancy was also reported in pediatric brain tumors (133) and a further study in pediatric brain tumors suggested the presence of lipids as a marker of poor survival (134). Taken together, the presence of MR-visible lipid signals seems to be a marker for malignancy and poor prognosis. In our recent review on the role of lipids in tumors (92) we argued that increased MR-visible lipids in tumors are indicative of a stress response and that their localization reflects areas of cellular hypoxia and or necrosis.

MRS methods have been used to assess changes in the metabolic profile of tumors as markers of early therapeutic response. Most xenograft and clinical studies have focused on evaluating reductions in the total choline or lactate signal as a marker of treatment response. Changes in lipid signals not only suggest a positive response, but increases in PUFA, a putative marker of apoptosis (106), may also aid in understanding the mechanism of cell death induced by specific treatments.

Gene therapy induced changes in lipids

One of the first studies to implement MRS to evaluate changes in metabolism during gene therapy reported a progressive increase in the lipid resonance at 1.3 ppm in 9L rat tumors that were injected intratumorally with adenoviral HSV-*to* followed by ganciclovir treatment (135). These initial studies were followed by several studies of the HSV-*tk* positive BT4C glioma model treated with ganciclovir (106,129,136), which established accumulation of PUFA resonances as a marker of treatment induced apoptosis. The PUFA resonances arise from phospholipase A2 activity leading to hydrolysis of phospholipids in the cell membrane (137). These studies indicate that early increases in mobile lipid resonances after therapeutic intervention may be suggestive of apoptosis and positive treatment response. The temporal evolution of these resonances may be variable, with a decrease in lipid resonances during formation of scar tissue after cell death and lysis. However, the mode and nature of therapeutic interventions could affect changes in lipid resonances. A recent study involving oncolytic viral treatment of immunocompetent Syrian hamster carcinomas as well as a patient with neuroblastoma reported lower unsaturated fatty acids, as measured from the olefinic resonance at 5.3 ppm (Figure 3) in responding tumors than in non-responding tumors (138), which appears to be an exception to the norm. Unlike previous studies, which resulted in an apoptotic tumor cell death after treatment, treatment with oncolytic viruses resulted in multifocal necrosis and a substantial cellular inflammatory response. Taken together, these studies indicate that changes in PUFA resonances could possibly be used to differentiate apoptosis from necrosis, although further data is needed to substantiate this finding.

A decreased PUFA resonance, in comparison to normal breast tissue, was reported in a patient with breast cancer using selective multiple quantum coherence spectroscopy (139). In

contrast to most tumors, a decrease in total tumor lipids was observed in pancreatic cancer compared to pancreatitis as evaluated by MR studies of patient biopsy samples (140) as well as *in vivo* comparing focal pancreatitis to pancreatic adenocarcinoma (141). Although the exact reason for this apparent anomaly is unclear, the pancreatic tumor microenvironment includes stromal tissue that comprises up to 80% of the tumor mass (142). Thus, it is possible that the dominant factor contributing to the MR visible lipid signal in pancreatic cancer arises from the dynamic assortment of extracellular matrix components, infiltrating immune cells, macrophages, pancreatic stellate cells, vascular cells, fibroblasts and myofibroblasts.

Chemotherapy induced changes in lipids

There have been several MRS studies describing alterations in lipid levels in response to treatment with cytotoxic agents. Increased triglyceride resonances were observed in the malignant MDA-MB-435 breast cancer cell line treated with the anti-inflammatory agent indomethacin (143), a drug that has been shown to reduce tumor invasion and inhibit metastasis. Similar increases have also been observed in cells treated with antimetabolic agents and other cytotoxic drugs (96,144-148). Treatment of DU145 prostate cancer cells with the differentiating agents phenylacetate or phenylbutyrate also led to a time dependent increase in lipid signals, accompanied by increased in GPC, indicating cell stress that precedes the induction of apoptosis (107,147). Increased apoptosis in BT4C glioma cells after exposure to cisplatin resulted in increases in both saturated fatty acids and PUFA resonances (149). **Treatment with subcytotoxic doses of cisplatin induced similar increases in saturated and total unsaturated fatty acid (5.3 ppm) resonances in HER2+ ovarian cancer cells (150) (Figure 4).** Exposure of the VEGFR tyrosine kinase inhibitor SU1498 on human U87 glioma cells induced a significant increase in lipids with a concomitant decrease in GPC (151). Although cell studies point towards

an increase in MR-visible lipid signals in tumors as a marker of response to chemotherapy, translation of these findings to *in vivo* settings have been challenging due to the confounding presence of lipid signals from hypoxic/necrotic regions in the tumor or contamination from lipid signals outside the tumor.

Many factors influence *in vivo* MRS and make it difficult to predict whether it will provide useful biomarkers for new therapeutic interventions. For example, the PI3K/mTOR inhibitor voxali in the presence or absence of temozolomide directly alters the phosphorylation of the phospholipid PtdIns in glioblastoma tumors, but causes no detectable changes in ^1H MR spectra, even though hyperpolarized ^{13}C MRS showed reduced conversion of pyruvate to lactate (152). Nevertheless, many studies have successfully evaluated changes in lipid signals in response to chemotherapy in tumor xenografts. The efficacy of etoposide, a topoisomerase inhibitor, was tested on a murine lymphoma model and a significant increase in the 1.3 ppm methylene mobile lipid resonance was noted following etoposide-induced apoptosis (153). Treatment of F98 rat gliomas with the choline kinase inhibitor MN58b resulted in a significant increase in both saturated (1.3 ppm) and polyunsaturated (2.8 ppm) fatty acids (Figure 5) that correlated with increased treatment-induced apoptosis (114). Similar increases in saturated and polyunsaturated lipid resonances were observed in GL261 glioma tumors in mice treated with temozolomide (128) (Figure 6). In contrast, treatment of C6, RG2, and GL261 glioma models with the nitrones, PBN (α -phenyl-tert-butyl nitron) and OKN007 (2,4-disulfophenyl-PBN) resulted in a decrease in lipid resonances after treatment, which was attributed to a decrease in necrosis and normalization of the metabolic profile rather than the increase in apoptosis generally reported in tumor treatment studies (154). Increases in neutral lipid resonances were also correlated with tumor growth arrest and treatment response in a mouse model of HER2+

ovarian cancer treated with cisplatin (150). No changes in total choline were noted in this study, as the authors found changes in neutral lipids to be a more sensitive marker of treatment response.

Radiation therapy induced changes in lipids

Radiation therapy is one of the most commonly used therapeutic strategies for solid tumors in the clinic. Although several studies point towards increased lipid resonances in radiation-induced necrosis, relatively few studies have evaluated changes in lipid resonances as a marker of early response to radiation therapy. Cervical cancer biopsy samples obtained from patients treated with radiation therapy were studied by HR-MAS, which revealed a direct correlation between the degree of apoptosis and lipid resonances (155) confirming the observations made in cell and xenograft studies. A xenograft model of non-Hodgkin’s diffuse large B cell lymphoma treated with radiation therapy demonstrated a significant increase in both mono and unsaturated lipid resonances within 3 days of treatment (156). The increase in lipid resonances corresponded with histology findings of increased apoptosis and oil red O staining, supporting the idea that unsaturated (PUFA) lipid accumulation is a marker of therapy induced apoptosis.

D. Positron Emission Tomography

Positron emission tomography (PET) measures the gamma rays produced when an electron collides with a positron emitted from a beta-decaying nucleus. PET scans require the administration of a radioactive substrate, but the high sensitivity of PET means that only tracer levels of radiolabeled material are needed. For tumor imaging, ¹⁸F-fluorodeoxyglucose (FDG) is by far the most commonly employed PET tracer (Figure 7). FDG exploits the enhanced aerobic glycolysis observed in many tumors, a phenomenon known as the Warburg effect.

Overexpression of plasma membrane glucose transporters and hexokinase activity rapidly internalize FDG and phosphorylate it in the first step of glycolysis. This reaction traps FDG-6-P in the cell, but the substrate is unable to be metabolized further. FDG-PET is widely used clinically for the staging of primary tumors, but because of the ability of PET to perform a full-body scan, it is also important for the detection of metastases. FDG-PET can also be used to monitor tumor regression post-therapy, and has been used in several instances to monitor FASN inhibitor therapies due to the link between glycolysis and fatty acid synthesis (157,158). One caveat is that it is difficult to assess enhanced FDG tumor uptake in tissues with high glucose utilization (e.g. brain and heart) or in organs of excretion (e.g. kidney and bladder). There is also the potential for background labeling in metabolically active cells, such as lymphocytes in sites of inflammation. Furthermore, some tumors, such as the prostate, are not FDG avid and have relatively low uptake.

A promising alternative to FDG for tumor lipid imaging is ^{18}F or ^{11}C labeled choline (Figure 7). This strategy relies on the upregulation of choline transporters and ChoK in tumor cells. The concerted action of these components achieves tumor tissue contrast by rapidly internalizing and phosphorylating the choline mimetic radioligands. Choline PET is becoming more widely used for tumor detection, especially in the prostate (159) but also in other tumors such as gliomas (160). Choline PET has also been used to track tumor recurrence, metastasis, and response to chemo and radiotherapy (161-164). One challenge facing choline PET is the discrepancy between labeled choline tracer uptake and steady state metabolites observed using MRS (165). This could be due to intrinsic differences between choline transport and phosphorylation that manifest as differences in tracer uptake relative to steady-state levels, but it

could also be due to alternative pathways such as metabolism of the choline tracers in other organs such as liver (166).

The low FDG avidity of prostate tumors is linked to their metabolic preference for β -oxidation of fatty acids, rather than glutaminolysis or glucose metabolism, as a primary energy source (42). A variety of ^{18}F -labeled fatty acid analogs have been synthesized (Figure 7), and the metabolism of these probes effectively trap the radiolabel specifically in cells with high usage of β -oxidation (167,168). In metabolizable probes, labeling at the hydrophobic end of the molecule allows these probes to be consumed through β -oxidation. In non-metabolizable probes, chemical modifications are nearer to the carboxyl group, which still allows cell uptake and potential incorporation into phospholipids or triglycerides, but further oxidative metabolism is prevented (169). Labeled fatty acid probes, such as BMIPP (Figure 7), have been primarily employed to monitor metabolism in tissues with high levels of β -oxidation, mostly cardiac and to a lesser extent skeletal muscle (170-176). Use of these probes to study cancer has been limited, even though the use of β -oxidation as a potential cancer energy source has been known for decades (177,178).

Although the half-life of ^{11}C is quite short (20 min), it is still possible to observe ^{11}C palmitate uptake and distinguish storage in triglycerides vs metabolism by β -oxidation (179,180). Regions of high fatty acid synthesis have been imaged using ^{11}C -acetate incorporation into palmitate (181), although the intermediate acetyl-CoA has other potential metabolic fates such as the Krebs cycle or histone modification. For this reason, ^{11}C -acetate has been used to monitor prostate tumors during therapy and recurrent lesions, but is incapable of distinguishing malignant tumors from benign hyperplastic nodules (182-185). Several radiolabeled ether lipids containing PC moieties (186) have been made (Figure 7) based upon early observations of elevated

phospholipid-ethers in neoplastic tissue (187,188). Due to the preferential accumulation and retention of these alkylphosphonium analogs, ^{124}I -CLR1404 was developed and recently tested in brain tumor PET imaging where a tumor/normal brain signal ratio of 30 was reported (189). Replacement of the PET isotope with the radio-ablative ^{131}I -CLR1404 agent improved survival in tumor-bearing rodents and provided a novel theranostic platform wherein ^{124}I can assist in the planning of ^{131}I therapy.

E. Mass Spectrometry Imaging

Mass spectrometry imaging (MSI) is a powerful modality for the detailed spatial detection of protein, metabolite and drug distribution in tissues. MSI consists of a family of techniques that includes matrix-assisted laser desorption ionization (MALDI) MSI, secondary ion mass spectrometry (SIMS), matrix electrospray-MSI (ES-MSI), and nanostructure-initiator mass spectrometry (NIMS). For the detection of lipids, the most commonly used technique is MALDI MSI (190), which has a large range of applications and provides a good overall combination of spatial resolution, intact molecule sensitivity and probing depth (190). MALDI provides a critical platform for the spatial detection of lipids and lipid metabolites, as there is a lack of suitable reagents and antibodies for the analysis of tissue lipids *in situ* (191).

MSI techniques are invasive procedures, requiring the preparation and embedding of a thin tissue slice followed by rasterized destruction by laser or primary ion beams. For MALDI, tissue is embedded in a matrix such as gelatin and α -cyano-4-hydroxycinnamic acid (CHCA) or 2,5-dihydroxybenzoic acid (DHB) (192). Washing the tissues with aqueous solutions of ammonium formate or phospholipases prior to embedding can be used to enhance certain lipid species and improve signal to noise (191). MSI can detect hundreds of molecules in a single sample with a routine spatial resolution of 50–100 μm , and a small molecule resolution as high

as 5–10 μm (193). Because matrix deposition is a slow process and the application of a wet matrix to tissue can cause metabolite shift, matrix-free techniques using nanoparticle initiators have been developed and these often provide simpler spectra to interpret (194,195). These NIMS procedures provide comparable lipid profiles, but with improved spatial resolution (196).

MSI has been used extensively to study lipid in primary human tumor tissue as well as tumor cell lines and xenografts. In human tissues, a consistent observation of elevated phospholipids has been observed in cancer compared to normal tissues. This includes breast tumors and invasive ductal carcinomas compared to carcinoma *in situ* (197-199), gastric cancers (200), renal cell carcinomas (201), human lung squamous cell carcinoma (202) and colorectal carcinoma (203,204). The most frequent observation is of increased PtdCho in tumors, but increases in PtdEtn, PtdIns, and PA have also been observed (199,205,206). Some studies have demonstrated increases in ether-linked lipids (199). Lyso-phospholipids have been reported to increase in colorectal tumors, but decrease in gastric and prostate cancers (200,203,207) (Figure 8). Fatty acid levels have also been observed to increase in a number of tumors (199), but it is uncertain whether these are free fatty acids or acyl components of more complex lipids.

Studies in tumor xenografts have confirmed the general observations of increased phospholipids and ether-linked lipids in tumor tissues. Measurement of choline metabolites in breast cancer xenografts using MR spectroscopic imaging combined with MALDI-MSI revealed differences in spatial distribution with PC and choline levels concentrated in normoxic tumor regions (208). When correlated with the expression of a hypoxia-response element, PtdCho elevation was concentrated in hypoxic regions whereas LPC was elevated in necrotic regions (192,209). Further studies have indicated distinct microenvironmental lipid distribution with higher levels of ether-linked PtdEtn in viable tumor and ether-linked PtdCho in necrotic regions

(193). Elongated fatty acyl chain length in phospholipids of human lung squamous cell carcinomas was reproduced in mouse xenografts and correlated with the expression of the acyl chain elongase ELOVL6 (210). These data demonstrate the role of MSI in identifying distinct tumor microenvironments by their molecular signatures.

While MSI has revealed a number of potential lipid-related cancer markers, there are very few studies that have employed MSI lipids to monitor cancer treatment. One recent study has identified transient decreases in serum PtdCho and lyso-PtdCho associated with response to radiation in head and neck tumor patients (211). A second study has indicated that LPC (16:0) is a significant predictor of PSA recurrence in human prostate cancer patients (207). The NIMS technique is capable of simultaneous monitoring of drug accumulation and endogenous water-soluble metabolite distribution (212,213). Adopting this technique to study drugs that target lipid metabolism in tumors should be a priority in this field. These data indicate that further investigation into the utility of lipid biomarkers to assess treatment response is warranted.

F. Optical Imaging

Optical imaging is a cost-effective and relatively easy-to-use imaging modality that makes it a valuable tool for non-invasive longitudinal imaging. The range of detectable wavelengths is broad enough to allow multiplex imaging, and these principles have been applied to a number of medical diagnostic platforms, including flow cytometry, fluorescence microscopy, and diffuse optical imaging. *In vivo*, optical imaging is limited by attenuated light penetration through tissue due to scattering and absorption in the visible wavelengths. This is partially alleviated by using chromophores that absorb or emit in the near infrared region (675 – 900 nm) where absorption by hemoglobin, deoxyhemoglobin and water is at a minimum. In

addition, several endogenous proteins, such as laminin and elastin, autofluoresce and elevate the background signal during *in vivo* measurements. As a result, the observable depths of optical imaging are usually limited to 1 cm or less. Thus *in vivo* optical imaging has predominantly focused on single-channel fluorescence or bioluminescence on a macro level (< 15x magnification) in mice or other small animals. Bioluminescence is not practical in clinical settings, because it requires transfection with an invertebrate luminescent protein, but it is a highly sensitive reporter that, in the field of lipid metabolism, has been adapted to map regions of heightened fatty acid uptake (214). Advances in CCD sensitivity has recently sparked renewed interest in Cerenkov radiation as an additional source of emitted light that can be detected *in vivo* (215,216). This signal may one day offer functional information concordant with the tomographical information provided by PET (217).

New optical imaging technologies in oncology have largely followed advancements in vascular imaging. Before diffuse optical tomography became a subject of interest in mammographic screening, the observation was made that absorption of circulating hemoglobin changes in an oxygen-dependent manner (218). This was a pioneering study in translational optical imaging, but 20 years later routine clinical optical imaging remains limited outside of optical coherence tomography (OCT) in ophthalmic applications (219-221). OCT uses a rastered application of NIR light and interferometry to produce a 3-D scanning image of the retina. The use of NIR wavelengths still limits the application to surface phenomena, less than 1 cm deep in the tissue. OCT has also been employed in oncology, most notably to detect the development of esophageal tumors (222,223), With regards to lipids, OCT is most commonly used for atherosclerotic plaque detection in cadavers and patients, which is based upon its sensitivity to

lipid deposits in local vascular sites (224,225). This leads to the possibility for future applications of OCT as a method to distinguish lipid levels in treated tumor tissue.

In small animal optical imaging, enhanced contrast is often provided by the administration of exogenous NIR fluorescent compounds. There are a growing number of these molecular probes that allow for noninvasive optical imaging of lipid metabolism pathways. The probes are generally of three types: passive molecules that are used as blood pool agents and rely on leaky vasculature to accumulate at the site of pathology, targeted permanently fluorescent molecules that can be taken up selectively as substrates or inhibitors of lipid metabolic pathways, and smart quenched fluorescent molecules that can be selectively activated by enzymes. Fluorescence imaging using these probes allows for specific and localized detection of metabolic activity.

Indocyanine green (ICG) is a clinically approved blood-pooling agent that has been used for tumor detection. Collection of ICG in solid tumors has been attributed to the enhanced permeability and retention caused by disordered vasculature and insufficient lymphatic systems in tumors (226), however tumor-associated macrophages in lipid-rich regions may have an underappreciated role in this phenomenon. In atherosclerotic plaques, ICG has been observed to accumulate preferentially in the lipid-rich macrophages in both rabbits and patients (227). MRI, PET, and optical imaging of tumor-associated macrophages have been described extensively (228-230) and these methods should be used to explore co-localization of lipid profiles with immune infiltration in the tumor setting.

Lipid uptake and synthesis

Lipid uptake and synthesis are essential to the maintenance of mammalian cell membranes. For this reason, a constant source of precursors are required to maintain the

proliferative nature of the cancer cell. Tumor cells synthesize and accumulate membrane lipids, such as PtdCho, at a rate that greatly outpaces the surrounding normal tissue, and this offers an avenue that can be exploited for selective delivery of imaging and therapeutic agents. The introduction of fluorescently labeled lipid analogs into the tumor microenvironment can also be used to visualize lipid scavenger activity. Direct fluorescent labeling of PtdCho has been reported using visible fluorophores, so high background in normal tissues is a general feature of these approaches (231). A common approach is to employ fatty acid probes to which non-polar dyes such as BODIPY have been attached (232). Depending on the metabolic state of the cell, these lipid analogs can be incorporated into phospholipids or neutral lipids in lipid droplets. The alkylphosphocholine analogs described for PET imaging have also been adapted with BODIPY or heptamethine dyes for imaging of tumor margins and draining lymph nodes (233).

Choline kinase

Choline kinase catalyzes the conversion of choline to PC, and is elevated in several types of cancers. Its upregulation has been correlated with the transition of normal breast epithelium toward a malignant phenotype, and selective inhibitors have been developed for Phase I clinical trials in solid tumors (ClinicalTrials.gov, NCT01215864). Based on these studies, we developed a series of fluorescent inhibitors that selectively bind ChoK and emit fluorescence in the near infrared optical window (234). The design exploited the structural similarities between ChoK inhibitors and cyanine dyes frequently employed for optical imaging, and did not rely on dye conjugation to existing inhibitor structures (235). The probe, JAS239, showed elevated uptake in tumors that overexpressed ChoK, and more importantly, had reduced uptake in tumors after chemotherapy (236).

Phosphatidylinositol signaling pathway

Many oncogenic signaling pathways are mediated by PtdIns, which makes these lipids attractive targets for cancer imaging probes. In order to interrogate the role of these phospholipids on cell signaling, Yoon *et al.* engineered a fluorescent PIP₂ sensor to study its role in membrane remodeling, regulation of membrane proteins, and regulation of the cytoskeleton (236-238). This probe was engineered with the visible fluorophore (2-dimethyl-amino-6-acyl-naphthaline) optimized for its lipophilic properties, limiting its utility for detection of lipid dynamics *in vivo*. Because IP₃ and DAG mediate the release of intracellular calcium and are activated by lipid metabolic pathways, optical probes based upon flu-4 or BAPTA can provide an indirect means of assessing these bioactive lipids (239-241). DAG can be evaluated directly using radiolabeling (242) or using optical techniques such as FRET to detect activated DAG intercalation in the plasma membrane (243).

Phospholipases

Phospholipases are catabolic enzymes that can be targeted with lipid based quenched optical probes that are activated by enzymatic hydrolysis (39). Upregulation of cPLA₂ has been observed in breast and prostate cancers (112), and elevated levels of PC and phosphoethanolamine have been attributed to PLC activation in breast cancer, ovarian cancer and melanoma (109). Activatable probes with phospholipid-based structures, a linker that regulates enzyme accessibility, a quenchable fluorophore or light-emitting compound, and a cleavable quenching domain have been designed as fluorescent agents for quantifying phospholipase activity. The advantage of this approach is the enhanced signal to noise available from the continuous enzymatic release of fluorescent substrates. Enzyme activated BODIPY probes have been employed to examine phospholipase activity in many systems including the

digestive physiology of the zebra fish (244-247). While useful for cells, small transparent animals and intravital microscopy, the optical properties of BODIPY limit its detection in tissue. There are several quenched fluorophores commercially available with emission in the visible range. In the NIR range, we developed analogous probes targeting PLA₂ and PLC for *in vivo* applications (248). These self-quenching probes were composed of pyropheophorbide-*a* (Pyro) tethered to PtdEtn and a Black Hole Quencher (BHQ-3). While Pyro has an ~~excitation maximum~~ ~~excitable-Soret band~~ at 418 nm that emits at 660 nm, its absorption peak at 670 with emission at 725 nm makes it suitable for *in vivo* imaging. The specificity of these probes to various phospholipase isoforms could be modulated by altering the spacer length between Pyro and the glycerol backbone. The resulting construct, Pyro-PtdEtn-BHQ, was highly specific to PtdCho-specific PLC (PC-PLC) both *in vitro* and *in vivo* and we used this to detect upregulation and activation of this enzyme in DU145 prostate cancer xenografts (Figure 9) (112).

Arachidonic acid, a PUFA found in the *sn*-2 position of phospholipids, is mobilized by the activity of cytosolic or cPLA₂ acting on membrane-associated phospholipids. The redox activity of COX-2 catalyzes the conversion of arachidonic acid to prostaglandins that act as a lipid second messenger. Arachidonic acid is first converted to prostaglandin G₂, and then the peroxidase activity of COX-2 converts it to the unstable H₂ isoform that is converted to one of several homologous tissue- and function-specific isoforms of prostaglandins. It is prostaglandin E₂ that is notably upregulated in several types of cancer contributing to aberrant signaling (249). In order to utilize this signal transduction pathway to image pathogenic events, there are several points of interrogation possible: using activatable probes to evaluate COX-2 activity, directly measure PGE₂ levels with targeted probes, or evaluate activation of further downstream events.

Detection of cPLA2 using a cleavable construct requires a probe that contains arachidonic acid, to account for the specificity of the enzyme for this fatty acid, as well as a small enough head group to fit into the active site. Previous studies had shown that arachidonic acid esterified to visible fluorophores such as 7-hydroxycoumarin were highly specific for cPLA2 and displayed similar hydrolysis kinetics to native substrates (250). We adapted this caged fluorescence protocol using fluorophores that emit in the red wavelength range (660-680 nm) to create cPLA2 sensitive probes suitable for *in vivo* imaging. Probe selectivity could be modulated by choice of fatty acids and fluorophores, with DDAO arachidonate performing the best *in vitro*, in cells and tumors. Here, arachidonic acid provided the optimum fatty acid substrate for cPLA2 and DDAO (7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one) was the most resistant to non-specific aqueous hydrolysis. Probe activation was shown to be proportional to cPLA2 expression levels in cells and tumors, and high relative to a non-specific control probe, DDAO palmitate.

Apoptosis

In addition to using imaging techniques to noninvasively detect components of lipid metabolism, other biomarkers can be detected that offer information pertaining to non-homeostatic states of cells and tissues *in situ*. Apoptosis is often indicative of patient response to cancer therapy, accordingly, targeting markers of apoptosis can be useful to evaluate the efficacy of various interventions (251). Among these events are plasma membrane reorganization marked by PtdSer translocation to the outer leaflet of the membrane. PtdSer translocation can be detected using labeled ligands that bind anionic phospholipids, including proteins such as Annexin 5 or synaptotagmin (252) or organic molecules such as zinc dipicolylamine (253). A number of

labeling strategies have used this approach to create apoptosis sensors for optical (254), MR (255-257) and nuclear imaging when radiolabeled with ^{99}Tc or ^{123}I (258). Annexin has shown some promise as a molecular probe for this purpose though this strategy can be limited by suboptimal pharmacokinetics (259), and non-specific labeling of necrotic cells (260).

Conclusion

Lipid metabolism is an essential component of cellular homeostasis that can become drastically altered during the process of malignant transformation. Most common driver mutations and oncogenes are related to growth cues and cellular stress responses, and many therapeutic strategies target these pathways such that the measurement of lipid metabolic changes can serve as a proxy for drug response pharmacodynamics. While MRS and PET approaches offer the ability to serially-measure lipid composition and flux, they are limited by spatial resolution. MSI and Optical Imaging, on the other hand, permit the appreciation of intratumoral heterogeneity but often require surgical exposure of the tissue site. Still, these approaches are finding increasing value in image-guided surgical resection and are experiencing fast-paced improvements in technology including the ability to image endogenous contrast, more specific probes that provide kinetic information, and sub-cellular resolution. For a balance between resolution and ability to image endogenous lipid signals without the need for new FDA-approved contrast agents, MRS remains the gold standard for measuring lipid metabolic states in tumor and other tissues. New advances including two-dimensional MRS, hyperpolarized MR spectroscopic imaging, and higher field strength MRS continue to reshape our understanding of lipid metabolic changes during tumor growth and treatment response. This comes at a critical time as therapy continues to evolve with new signal transduction inhibitors, metabolic targeting agents, and immunotherapies change the landscape of cancer care.

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Figure Legends

Figure 1: Lipid metabolism pathways in the cell. Glucose transported into the cell is metabolized to pyruvate via the glycolytic pathway, and can be fed into the tricarboxylic acid (TCA) cycle for ATP production in the mitochondria. Citrate from the TCA cycle or from exogenous sources is converted to Acetyl-CoA in the cytoplasm and can be used for cholesterol or fatty acid (e.g. palmitate) biosynthesis. Cholesterol produced in this fashion can be used to synthesize membranes or be incorporated into sterols. Fatty acids can be desaturated or extended and then incorporated into triglycerides or incorporated into phospholipids via the Kennedy Pathway. Cholesterol and triglycerides fatty acids can also be taken up through specific transport mechanisms. The resulting lipid droplets, shown in the inset, contain fatty acids stored in phospholipid and fatty acid form that can be mobilized by phospholipases and lipases, respectively. Beta-oxidation (β -Ox) of free fatty acids from lipid droplets can be triggered to meet energy needs. In place of the third acyl group (Acyl_3) attached to the glycerol moiety of triacylglycerides (orange oval), phospholipids have a phosphorylated polar head group (yellow sphere) that confers water solubility and creates organized bi-layer and micelle structures. Phospholipases can also elicit 2nd messenger signaling cascades by releasing bioactive fatty acids (e.g. sphingolipids, eicosanoids) and/or polar head groups in response to growth or stress cues. External cell signaling cues can also be transmitted by lipids in the case of isoprenylation and subsequent transport of small GTPases to dock with G protein-coupled receptors (GPCRs) on the cell surface.

Figure 2: Major lipid metabolic pathways altered in cancer cells. Metabolic reprogramming during malignant transformation mimics autonomous growth signaling in unicellular organisms. Common oncogenes in the PI3K/Akt/MTOR pathway, are often mutated

or upregulated due to their presence downstream of external growth factors and their ability to re-route cellular carbon sources toward fatty acid utilization as an energy source. Cancer cells can also supply the TCA cycle from glucose via aberrant glycolysis and from glutamine via IDH mutations, although the contributions of these pathways differ greatly depending on the energy needs of the cell. The transcription factor Myc promotes many malignant processes in cancer cells because it is a master regulator capable of triggering survival pathways in response to hypoxia and nutrient deprivation. Hypoxic conditions are a factor in many solid tumors and influence the local tumor microenvironment by dampening the immune response and activating hypoxia-inducible factor-1 α (HIF1 α), which among other things, initiates the regulatory function of sterol regulatory element binding protein 1 (SREBP-1) on fatty acid synthesis. Free fatty acids such as palmitate are used as energy sources or converted to bioactive eicosanoids to dampen the immune response and stabilize the ER stress that can result from saturated fatty acid accumulation.

Figure 3: Changes in lipid metabolism in response to oncolytic viral treatment of Syrian hamster carcinomas. The oncolytic viral treatment induces coagulative necrosis, which is seen as hypo-intense areas within the tumor on T_2 -weighted MR image. *In vivo* MRS from the voxel (overlaid on the image) is shown on the right demonstrating resonances from unsaturated fatty acids, taurine and choline. ~~Unlike the increased PUFA resonances observed during apoptosis (Figure 3), no~~ No changes in PUFA resonances were observed in coagulative necrosis resulting from oncolytic viral treatment. Reprinted with permission from (138).

Figure 4. A) Confocal micrographs of SKOV3.ip cells treated for 48 h with cisplatin (CDDP) show increased lipid droplets as measured by Nile red staining, compared to untreated controls (CTRL). Fixed cells were counterstained with phalloidin 488 for actin (green) and DAPI for nuclei (blue). Scale bar represents 23.8 μm . (B) ^1H NMR spectra of intact SKOV3.ip cells treated with 5 mM CDDP for 48 h (red) show increases in mobile lipids compared with untreated control cells (black). Labeled lipid resonances include: methine protons at 5.3 ppm ($-\text{CH}=\text{CH}-$); fatty chain methylene group at 1.3 ppm ($-(\text{CH}_2)_n-$); methyl group at 0.9 ppm ($-\text{CH}_3$); and total choline (tCho) at 3.2 ppm ($-\text{N}(\text{CH}_3)_3$). Reprinted with permission from (150).

Figure 5: Increased lipids in response to choline kinase inhibition as an alternate therapy for the treatment of gliomas. *In vivo* MRS from an untreated F98 rat glioma (bottom spectrum) and after 5 days of treatment with a choline kinase inhibitor, MN58b (top spectrum). Increased mono-unsaturated lipid peaks (1.3 ppm) are evident with treatment. In addition, a significant increase in the poly-unsaturated fatty acids (PUFA, 2.8 ppm), indicating apoptotic cell death. As expected, a decrease in the total choline (tCho) peak was also observed in response to choline kinase inhibition. The MR image demonstrates placement of the voxel for MRS studies. Reprinted with permission from (114).

Figure 6: Single voxel ^1H MR spectra from a GL261 tumor implanted into a mouse brain. Treatment with three cycles of temozolomide led to significant (*) increases in mobile lipid:Cre and tCho:Cre resonances at 28 days post inoculation (left) compared to spectra acquired before treatment (right). Labeled resonances: Cho (choline: 3.2 ppm, Cre (creatine): 3.3

ppm, MLs (mobile lipid methylenes): 1.3 ppm, PUFAs: 2.8 ppm. Reprinted with permission from (128).

Figure 7: Chemical structure of the common PET tracers used for detection of lipid metabolism. The radioactive isotope is denoted in red.

Figure 8: MALDI-MSI images and corresponding H&E sections from human colorectal tumors (A-C) and adjacent tumor free regions (D-F). A selective projection of m/z 478.3 onto MALDI-MSI images reveals elevations in 1-palmitoyl-lysophosphatidylcholine in cancer-containing areas compared to the non-tumor bearing tissue sections. Elevations in m/z 504.3, representing 1-oleoyl-lysophosphatidylcholine, were also observed in this study. Reprinted with permission from (203).

Figure 9: *In vivo* NIR fluorescence imaging of phospholipase activity using the PC-PLC activatable probe Pyro-PL-BHQ in DU145 prostate tumor xenografts. Each mouse received 80 nmol *i.v.* of (A) Pyro-PtdEtn, a permanently fluorescent analog used as a control, (B) Pyro-PtdEtn-BHQ or (C) Pyro-PtdEtn-BHQ plus pre- and post- injections of the PC-PLC inhibitor D609. The fluorescence intensity from Pyro-PL-BHQ activation peaked at 6-7 h, and was inhibited by D609 treatment. Fluorescence persisted for up to 31 h in tumor tissue (T) excised from mice when compared to muscle (M) control. Reprinted with permission from (112).

Imaging of Cancer Lipid Metabolism in Response to Therapy

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Abstract

Lipids represent a diverse array of molecules essential to the cell’s structure, defense, energy, and communication. Lipid metabolism can often become dysregulated during tumor development. During cancer therapy, targeted inhibition of cell proliferation can likewise cause widespread and drastic changes in lipid composition. Molecular imaging techniques have been developed to monitor altered lipid profiles as a biomarker for cancer diagnosis and treatment response. For decades, magnetic resonance spectroscopy has been the dominant noninvasive technique for studying lipid metabolite levels. Recent insights into the oncogenic transformations driving changes in lipid metabolism have revealed new mechanisms and signaling molecules that can be exploited using optical imaging, mass spectrometry imaging, and positron emission tomography. These novel imaging modalities have provided researchers a diverse toolbox to examine changes in lipids in response to a wide array of anticancer strategies including chemotherapy, radiation therapy, signal transduction inhibitors, gene therapy, immunotherapy or a combination of these strategies. The understanding of lipid metabolism in response to cancer therapy continues to evolve as each therapeutic method emerges, and this review seeks to summarize the current field and areas of unmet needs.

Abbreviations

¹⁸F-fluorodeoxyglucose (FDG)

2,5-dihydroxybenzoic acid (DHB)

3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA)

acetyl-coenzyme A (acetyl-CoA)

acute myeloid leukemia (AML)

AMP-activated protein kinase (AMPK)

ATP-citrate lyase (ACLY)

beta-oxidation (β -Ox)

black Hole Quencher (BHQ-3)

choline kinase (ChoK)

choline transporters (ChoTs)

cisplatin (CDDP)

7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one) (DDAO)

diacylglycerol (DAG)

(4',6-diamidino-2-phenylindole) (DAPI_

elongation-of-very-long-chain-fatty acids (ELOVL)

endoplasmic reticulum (ER)

epidermal growth factor receptor (EGFR)

fatty acid binding protein-4 (FABP-4)

G-protein coupled receptor (GPCR)

glycerophosphocholine (GPC)

hormone-sensitive lipase (HSL)

hypoxia inducible factor (HIF)

Indocyanine green (ICG)

isocitrate dehydrogenase-1 (IDH1)
lipoprotein lipase (LPL)
low density lipoprotein (LDL)
lysophosphatidylcholine (LPC)
magnetic resonance spectroscopy (MRS)
mass spectrometry imaging (MSI)
matrix-assisted laser desorption ionization (MALDI)
mono-unsaturated fatty acids (MUFAs)
nanostructure-initiator mass spectrometry (NIMS)
OKN007 (2,4-disulfophenyl-PBN)
optical coherence tomography (OCT)
PBN (α -phenyl-tert-butyl nitron)
peroxisome proliferator-activated receptor alpha (PPAR α)
phosphatidylcholine (PtdCho)
phosphatidylethanolamine (PtdEtn)
phosphatidylinositol (4,5) phosphate-2 (PIP2)
phosphatidylinositol (PtdIns)
phosphatidylserine (PtdSer)
phosphocholine (PC)
phosphoethanolamine (PE)
Phosphoinositides (PI)
phospholipase (PL)
phosphomonoester (PME)
plasma membrane citrate transporter (PMCT)
polyunsaturated fatty acid (PUFA)

positron emission tomography (PET)

protein kinase C (PKC)

PtdCho-specific PLC (PC-PLC)

pyropheophorbide-a (Pyro)

reactive oxygen species (ROS)

secondary ion mass spectrometry (SIMS)

sphingosine-1-phosphate (S1P)

stearoyl-CoA desaturase-1 (SCD1)

sterol response element-binding proteins (SREBPs)

total choline (tCho)

tricarboxylic acid (TCA)

unfolded protein response (UPR)

α -cyano-4-hydroxycinnamic acid (CHCA)

1
2
3 **Introduction**
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5 Cancer cells exist in a hyperactive state of growth and use a complex network of lipid
6 metabolic pathways to support this growing biomass. Lipids are a broad class of compounds that
7 include fatty acids, triglycerides, steroids, phospholipids, and sphingolipids, among others. These
8 molecules play critical roles in cellular compartmentalization, structural barriers, communication
9 signals, energy storage, and homeostasis. Despite high vascularization, many cancer cells exist in
10 regions of nutrient deprivation and tumors have adopted alternative strategies to maintain sources
11 of lipid. In some cases the altered lipid metabolic state offers a therapeutic vulnerability, while in
12 others, adaptations to intracellular lipid composition can be used by refractory tumors to resist
13 therapies. Because these cancer-driven aberrations in lipid metabolism often contrast the
14 surrounding tissue, there are several molecular imaging strategies that have been developed to
15 monitor tumor margin, stage, and treatment response.
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32 **I. Lipid Metabolism**
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34 **A. Exogenous Lipid Uptake**
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36 *De novo* lipid synthesis pathways are crucial during embryogenesis and fetal
37 development, but, after maturation, cells in most tissues are capable of acquiring sufficient
38 circulating lipids to meet their biosynthetic and energetic needs (1-3). Cancer cells can access
39 circulating lipids by overexpressing lipid-scavenging proteins. High exogenous lipids are
40 associated with local invasive index, and obesity is also correlated with higher cancer incidence
41 rates (4). Higher expression of low density lipoprotein (LDL) receptor has been found in
42 transformed colorectal cells relative to normal cells (5). The LDL receptor in prostate cancer
43 provides a major source of cellular cholesterol and essential fatty acids (6). Breast cancer and
44 select sarcoma cells have been observed to secrete lipoprotein lipase (LPL) to release fatty acids
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from triglycerides in circulating lipoproteins (7). Although macropinocytosis has been identified in cancer cells, little evidence exists to suggest that this is a relevant source of exogenous lipid *in vivo* (8). Instead, lipid binding proteins are thought to assist in the capture of lipids from the interstitial space and promote invasion (Figure 1), as overexpression of fatty acid binding protein-4 (FABP-4) has been observed on the surface of ovarian, prostate, bladder, and renal cancer cells (9,10). Fatty acid translocase, also known as CD36, is a long-chain fatty acid scavenger that contributes to high mammographic density in subjects at high-risk for breast cancer (11). Ovarian cancer cells can siphon free fatty acids from the lipid stores of the omentum by activating perilipin-A and hormone-sensitive lipase (HSL) in neighboring adipocytes (12). Studies have shown that removal of lipids from culture medium can trigger *de novo* lipid synthesis, emphasizing the dependency on lipids for cell proliferation and the adaptations cancer cells make to sustain their growing biomass (13).

B. *De Novo* Synthesis

Many cancers revert back to *de novo* lipid synthesis, and the lipogenic pathway is composed of many enzymes that are critical for tumor growth (14). Lipid synthesis starts at the energetic hub of the cell, the mitochondria, where acetyl-coenzyme A (acetyl-CoA) and oxaloacetate are condensed into citrate (Figure 1). Citrate from the TCA cycle can be shunted from the mitochondria and broken down by ATP-citrate lyase (ACLY) into acetyl-CoA. Cytosolic citrate can alternatively be sourced from the microenvironment by plasma membrane citrate transporter (PMCT)(Figure 1) (15). Besides feeding into the rate-limiting step of fatty acid synthesis, acetyl-CoA is also critical for histone acetylation and serves as a link between metabolic status and gene expression (16,17).

Acetyl-CoA serves as the 2-carbon building block used for fatty acid synthesis. The enzyme FASN combines malonyl-CoA with repeated acetyl-CoA condensations to form palmitate. Palmitate is the saturated 16-carbon fatty acid from which most complex fatty acids in the body are derived (18). FASN overexpression is associated with poor prognosis in breast cancer (19) and FASN inhibitors derived from natural products (e.g. resveratrol) and synthetic molecules (e.g. orlistat) are being studied for their anti-neoplastic effects (20). Acetyl-CoA is also a synthetic precursor to 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) in the cholesterol biosynthesis pathway (Figure 1). Mevalonate is then produced by HMG-CoA reductase, the target of the lipid lowering drugs, the statins. The amplification of HMG-CoA reductase in many prostate tumors creates a growth dependency on cholesterol, and suggests a possible role for statins in treating these cancers (5,21). Mevalonate is also a precursor to farnesyl-diphosphate, which can further be processed to cholesterol, or used as a substrate for protein prenylation. Isoprenoids from the mevalonate cascade are critical for membrane anchoring and activation of the growth-related G-protein subunits Ras (farnesylated), Rho (geranylgeranylation), and many others (22) (Figure 1).

Fatty acids can be chemically modified in many ways to meet the diverse range of specialized functions required for cellular function (Figure 1). Stearoyl-CoA desaturase-1 (SCD1) produces mono-unsaturated fatty acids (MUFAs) from the saturated fatty acid chains sourced exogenously or from *de novo* synthesis. Saturated fatty acid accumulation in the endoplasmic reticulum (ER) triggers autophagy-induced apoptosis by activating the unfolded protein response (UPR) (23). By modulating MUFA to saturated fatty acid ratios intracellularly, SCD1 has a regulatory effect on cell survival and proliferation (24-26). The elongation-of-very-long-chain-fatty acids (ELOVL) enzymes in the ER are responsible for polyunsaturated fatty

acid (PUFA) synthesis (27), as well as the conversion of saturated and monounsaturated fatty acids into the very long chain fatty acids ($C > 18$) that serve as building blocks of sphingolipids when combined with ceramide head groups (28). Free fatty acids are stored as triglycerides in lipid droplets or packed into the cell membrane as sphingolipids, cholesterol esters, or phospholipids. Lipid droplets in breast cancer cells have been found to increase with increasing malignancy and to be enriched in polyunsaturated fatty acids, especially arachidonic acid (29)

Diacylglycerol (DAG) is an important lipid second messenger that also serves as a synthetic precursor for both membrane phospholipids and lipid droplet triacylglycerides (30-33). Major membrane phospholipids include phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer), among others, and vary in function, location, and relative abundance. The Kennedy pathway of phospholipid synthesis describes the addition of polar head-groups to the DAG backbone, and enzymes in this pathway are responsible for the accumulation of phosphocholine (PC) and phosphoethanolamine (PE) observed in many cancers (34). Choline uptake by choline transporters (ChoTs) and phosphorylation by choline kinase (ChoK) have, in particular, been consistently linked with increased invasiveness, drug resistance, and overall malignancy (35).

C. Lipid Mobilization and Usage

Phospholipid and triacylglyceride levels are maintained by the concerted actions of catabolic enzymes that mobilize fatty acids from lipid droplets and cell membranes (36). Fatty acid release from lipid droplet storage involves lipases that can support aggressive cancer phenotypes in a manner similar to the pro-tumorigenic effects of exogenous fatty acids (37). Fatty acid release from membrane phospholipids is carried out by the phospholipase (PL) enzymes. PLA₁ and PLA₂ cleave phospholipids at the *sn*-1 and *sn*-2 positions, respectively. The

PLA₂ family of enzymes are the primary producers of arachidonic acid and three major subgroups exist: calcium-dependent or cytosolic cPLA₂, calcium-independent iPLA₂, and secretory sPLA₂ (38). While cPLA₂ has a tumor-supportive role in many cancers, sPLA₂ is thought to have tumor suppressive-functions (39). In contrast, iPLAs are housekeeping enzymes, primarily responsible for mobilizing lipids to maintain membrane integrity and general cellular energy metabolism during homeostasis. PLC cleaves phospholipids at the bond between glycerol and phosphate and contains pleckstrin homology domains for anchoring and cooperation with G-protein coupled receptor (GPCR) signaling networks (40). When activated by growth factor receptors, PLD hydrolyzes the phospholipid head group (e.g. choline, ethanolamine, inositol, serine) to release PA and links growth factor signaling to cell proliferation (32).

Saturated free fatty acids can be broken down into acetyl-CoA to feed into the TCA cycle, and some cancers have been found to prefer fatty acid oxidation to pyruvate oxidation, even in sufficient glucose and oxygen environments (41). β -oxidation is an important energy pathway in prostate cancers, where glycolytic rates are low, and rapid citrate utilization requires a constant supply of acetyl-CoA (42). Peroxisome proliferator-activated receptor alpha (PPAR α) is a transcription factor whose activation triggers breakdown of very long chain fatty acids in the peroxisomes, and fatty acid oxidation in the mitochondria. A complex network of interactions with PUFAs, namely arachidonic and linoleic acids, regulates PPAR α activity (43). Aside from their direct signaling functions (44), arachidonic acid is an important substrates for cyclooxygenase, lipoxygenase, and cytochrome enzymes that catalyze the production of the eicosanoid family of bioactive lipids that have roles in innate immunity, inflammation, cardiovascular disease, and cancer (45,46).

II. Tumor Progression and Regression

A. Oncogenic Reprogramming

A common feature of cancer cells is the metabolic transition from oxidative phosphorylation to glycolysis, during which the rate of glucose consumption far exceeds the energy requirements of the cell (14). What initially appears to be wasteful energy utilization is in fact a method to feed glucose-derived pyruvate into biosynthesis, including fatty acids (Figure 2). Glutamine has been identified in some cancers as an alternative energy source that can enter the TCA cycle (47) and provide a carbon source for citrate production (Figure 2), however the dependency on glutamine for anaplerosis varies considerably even among cancers of the same tissue (48,49). This may be due, in part, to heterogeneity in the local tissue microenvironment causing gradients in nutrients, oxygen and signaling factors that influence the energetic state of cells.

Under hypoxic conditions, solid tumors can become dependent on unsaturated fatty acid uptake from serum to prevent ER stress that can arise when rapid proliferation is unmet by nutrient availability (50). These exogenous fatty acids can arise from lipolysis in stromal cells in a manner comparable to cachexia (23). The recent resurgence of interest in cancer metabolism has improved our understanding of the adaptations tumor cells can make when fuel availability is variable. These adaptations are only possible because the common driving mutations in cancers often have direct involvement in metabolic pathways.

Many of the clinically prevalent oncogenes are capable of altering lipid uptake, production, or consumption to gain a competitive growth advantage (Figure 2). The transcription factor hypoxia inducible factor (HIF) is a downstream effector of Myc and is tightly regulated by the tumor suppressor gene p53. HIF induces FASN induction and lipid droplet formation for

energy storage to support the tumor microenvironment (18,51,52). FASN requires the reducing agent NADPH, which is produced from the pentose phosphate pathway that often becomes upregulated during cancer reprogramming (18). There is further evidence in renal cell carcinoma models that HIF2 α -dependent lipid storage suppresses the ER stress response promoting tumor cell survival (53). Glutamine uptake in glioma and acute myeloid leukemia (AML) is often accompanied or driven by isocitrate dehydrogenase-1 (IDH1) mutations that reroute glutamine toward citrate to provide fatty acid and cholesterol precursors independent of the TCA cycle (Figure 2) (54,55). In hypoxic environments where glucose metabolism is diverted to anaerobic lactate production, mutations in mitochondrial IDH2 provide a continued source of citrate for lipid synthesis, by allowing reductive carboxylation of glutamine-derived α -KG (56). The oncometabolite 2-hydroxyglutarate is produced by many cancer-specific IDH1 and IDH2 mutations, and has widespread epigenetic effectors by altering DNA methylation (57-59).

AMP-activated protein kinase (AMPK) serves as an energetic sensor and master switch by inhibiting fatty acid synthesis in low energy states and stimulating consumption of fatty acids for energy in the mitochondria by β -oxidation (Figure 2). Sterol response element-binding proteins (SREBPs) (60) can activate transcription of genes involved in cholesterol and fatty acid synthesis and uptake, and are directly downstream of AMPK and the PI3K/Akt/MTOR signaling axis that includes many oncogenes (61). Epidermal growth factor receptor (EGFR) mutations are common tumor-driving factors that recruit phospholipases (62,63) and ChoK α (64) to the cell surface, leading to membrane remodeling and induction of mitogenic signals to drive cellular proliferation. Mitogenic growth signals can also come in the form of sterol-derived hormones (estrogens, progestogens, and androgens). Release of these sex hormones, their receptor expression, and the downstream signaling mediators are all common tumor-driving factors in

prostate, breast, endometrial and ovarian cancers (65). Farnesylation of Ras and geranylgeranylation of Rho GTPases are essential for downstream signaling through the phosphoinositol family of signaling lipids (66,67). Phosphoinositides (PI) comprise 10-20% of total cell phospholipids and among that fraction, phosphatidylinositol (4,5) phosphate-2 (PIP₂) and phosphatidylinositol-4-phosphate constitute approximately 0.2-1% (68-70). This class of membrane lipids plays an integral role in proliferation, apoptosis, metabolism and migration, and is pivotal to transmembrane signal transduction because it regulates the distribution of receptor tyrosine kinases, G-protein-coupled receptors, and adhesion molecules among others (71).

Metabolic disease is a risk factor for many cancer types, promoting insulin resistance, hyperactive growth signals, and evasion of apoptosis that help set the conditions for malignant transformation (72). Histological tumor grade in the breast is clinically correlated with phosphomonoester (PME) accumulation (73), such as PC and PE, detectable by MR spectroscopy (74,75). PC promotes survival and mitogenic signaling through downstream cascades such as MAPK and Akt (73,76,77). PC can be formed through the actions of the enzyme choline kinase, or by phosphatidylcholine specific phospholipase C. Phospholipid catabolism can also be a source of other mitogenic second messengers such as DAG, PA, and lysophosphatidylcholine (LPC). DAG is released following the cleavage of phospholipids by PLC (78), activates protein kinase C (PKC) resulting in the release of intracellular calcium stores and subsequent Ca²⁺-dependent signaling. DAG and PC are also the synthetic precursors to the most abundant mammalian membrane phospholipid, PtdCho, whose levels are predictive of breast tumor grade, estrogen receptor status, and patient survival (79). It is intuitive to suspect that cancers in high adipose tissue use altered lipid metabolism to take advantage of the surrounding environment, however evidence of altered lipid metabolism in cancers that arise in

low-adipose tissues suggests that deranged lipid metabolism is a universal hallmark of cancer pathogenesis.

Variations in lipid composition are likely due to the heterogeneous nature of the tumor microenvironment, which is an ever-changing compartment of structural fibers, nutrients, cellular waste, and signaling molecules. Interstitial pH, extracellular matrix, bioactive lipids, and cytokines can alter the population and function of stromal cells and surrounding tissues. Extracellular lactate, the metabolic byproduct of aerobic glycolysis, is capable of transitioning tumor-associated macrophages from tumor-suppressive to tumor-supportive phenotypes (80,81). Biopsies of colon cancer specimens have been reported to contain high lipid deposition in regions of necrosis and infiltrating macrophages (82). These macrophages most likely resemble the immune-suppressive “alternatively-activated” phenotype, as classic (interferon gamma-induced) activation does not enhance lipid phagocytosis in human myeloid populations (83). Alternatively-activated macrophages prefer fatty acid oxidation (84) and are thought to have critical roles in clearing/remodeling necrotic tissue, recruiting new blood vessels, and regulating innate and adaptive immunity (85).

B. Lipid changes during Apoptosis/Necrosis

The role lipids play in nearly all cell survival pathways emphasizes their importance in tumor growth and invasion pathways, but lipid compartmentalization, metabolism, and signaling are also intricately involved in cell death pathways. During apoptosis, the release of lysophosphatidylcholine (LPC) and sphingosine-1-phosphate (S1P) by PLAs (86) and ceramidases (87) respectively, acts as a “find-me” signal to surrounding macrophages (88). Once recruited to the dying cell, exposed PtdSer on the apoptotic cell’s surface serves as the “eat me”

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3 signal recognized by phagocytes (89). Unlike the organized compartmentalization of apoptotic
4 cells into non-immunogenic apoptotic bodies, necrosis is characterized by the breakdown of the
5 plasma membrane and release of the cellular contents that often triggers an immune reaction
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7 (90). Recently, lipid peroxidase networks have been identified as key mediators of cancer cell
8 therapy resistance by reversing a non-apoptotic form of cell death known as ferroptosis can be
9 induced by lipid peroxide accumulation (91). Tumor cell response to therapy begins with
10 activation of cell stress responses, which can ultimately decide the fate of the cell and
11 surrounding tumor.
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21 Early changes in lipid metabolism have been reported in response to a wide range of
22 cellular stresses (92), but lipid mobilization does not necessarily commit a cell to apoptosis (93).
23 Many cells undergoing apoptosis produce lipid droplets, although there are exceptions (94), and
24 fatty acids are first mobilized from membrane phospholipids by PLA₂ activity (92,95). Reactive
25 oxygen species (ROS) produced in the mitochondria of apoptotic cells are thought to inhibit the
26 catalytically-active thiol groups on β -oxidation enzymes (96), thus free fatty acids are redirected
27 into lipid droplets in the form of triglycerides and sterol esters (97,98). Lipid droplet formation in
28 cancer may act as a drug reservoir to reduce intracellular concentrations of drug (99), or to
29 absorb reactive oxygen species to protect further DNA damage (100). Lipogenesis of saturated
30 fatty acids also makes the cell membrane less penetrable to drugs that enter by passive diffusion
31 (101). Uptake of triglycerides by macrophages or neutrophils can dampen subsequent immune
32 activation (102,103), providing another potential mechanism of immune escape. Ceramide
33 accumulation regulates and can even trigger mitochondrial outer membrane permeability to fully
34 commit a cell to apoptosis by allowing cytochrome C release to the cytosol (104). Other signs of
35 cell stress that precede or arise during apoptosis are the rise in PUFA and glycerophosphocholine
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(GPC) levels (105-107). These indicate a reprogramming of lipid metabolism and suggest that catabolic networks mobilize lipids from membrane phospholipids during this time. Many of these observations were first made using magnetic resonance spectroscopy (MRS) to non-invasively measure lipid resonances of cancer cells undergoing stress responses and apoptosis (92).

III. Imaging Metabolic Lipid Changes

The development of modern imaging techniques allows the detection of several key signaling pathways involved in lipid metabolism and its regulation/dysregulation in cancer. The ability to detect these pathways non-invasively aids in the discovery of potential targets for imaging or interventional therapies. In addition to developing probes to image lipids and alterations in lipid metabolism in mechanisms of oncogenesis, we can also image lipid-dependent or –mediated signaling in response to therapy (108).

A. Imaging lipids in tumors: MR Spectroscopy

MRS has been extensively employed for the study of biological material (from cell extracts, to homogenized tissue, and intact organisms) because it offers a non-invasive method to study the native distribution and dynamic nature of many relevant biomarkers of disease and therapeutic response. The search for disease-related metabolites requires the suppression of the overwhelming fat and water signals observed in proton MR spectra, however much can be learned from the diverse species that contribute to these lipid peaks.

B. Choline and choline metabolites

MRS has been essential in the study of lipid metabolism and for the non-invasive observation of lipid metabolism *in vitro* and *in vivo*. This is due to the ability to observe a number of lipid metabolites in ^1H and ^{31}P MR spectra on the basis of chemical shift discrimination. Due to the relative insensitivity of MR, observation is limited to soluble or MR-visible metabolites in the high micromolar to millimolar levels. Lipid metabolites observable in ^{31}P MR spectra, include the PME, PC and PE and the PDE, GPC and GPE. In ^1H spectra, observable lipid metabolites include the total choline (tCho) resonance, a composite resonance encompassing free choline, PC and GPC. Thus there is overlap in the information available between ^{31}P and ^1H spectra, since the choline PC and GPC resonances seen in the ^{31}P spectra are also observed in the tCho region of ^1H spectra. The PME and PDE resonances report on pathways relevant to phospholipid metabolism. PC and PE are generally the anabolic products of choline kinase and ethanolamine kinases, although they can also be produced by the actions of phospholipase C. The PDE resonances result from catabolism of phosphatidylcholine and phosphatidylethanolamine via the consecutive action of two phospholipases, A2 and lysophospholipase or A1. There are numerous excellent reviews on this topic (35,92,109,110), and the reader is referred to these for detailed information. However, it is worth noting that consistent elevations in PMEs and tCho have been observed in a wide range of tumors including brain, prostate, lung, skin, ovarian and breast (111,112). As a means to monitor therapeutic response, tumor choline levels have been measured using MRS in animal (113-115) and human tumors (116-120) with mixed results, at least partly due to the technical difficulties associated with acquiring choline spectra on a background of high fat in normal breast tissue. The picture is further complicated because ChoK is not the only enzyme that contributes to PC accumulation, it

can be produced directly by the actions of PLC on PtdCho, or by the hydrolysis of GPC as a source of additional choline for subsequent phosphorylation. Recent MRS applications have been used to detect changes in choline metabolism due to IDH mutations in glioma (59,121) explore the role of the glycerophosphodiesterase genes GPD5 and GPD6 on breast cancer cell migration/invasion (122) and profile metabolic changes in response to HIF1 and HIF2 suppression (123).

C: Imaging lipids in tumors: MR-visible lipids

Proton MRS was originally used to detect MR-visible or mobile lipid signals in cultured cells and tumor biopsies. A series of resonances were observed arising from the fatty acryl chains in neutral lipids, triglyceride and cholesterol esters, including the terminal methyl groups, the methylenes in long chain fatty acids, and olefinic MUFA/PUFA resonances (124-127). MR detects only molecules that exist in a local environment with high rotational molecular motion and thus the observed signals arise predominantly from mobile or MR-visible lipids, composed of triglycerides and cholesterol esters sequestered in lipid droplets. In animal and human tumors, the observation of these lipid resonances require the use of short echo time spectroscopic pulse sequences due to the relatively short T_2 relaxation times of mobile lipids. *In vivo* MRS studies initially used single voxel localization methods such as STEAM and PRESS for assessing the MRS pattern of lipids in tumors, due to the simplicity and accuracy of these methods. However, multi-voxel spectroscopic methods including chemical shift imaging are increasingly being used both in mouse glioma and xenograft models (128,129) as well as in human tumors (130) since these methods provide a better assessment of tumor heterogeneity. The presence of intense lipid signals from surrounding subcutaneous fat and muscle have been problematic in evaluating lipid signals by MRS methods, thus outer volume fat suppression sequences become critical. For this

reason, the majority of studies on intra-tumoral lipids have been focused on the brain where subcutaneous fat is at a minimum. However, useful insights into human tumor composition have still been made in other tumors, despite the problems associated with high fat content in peripheral tissue.

The presence of lipids in human tumors was initially reported by Kuesel *et al.* (124) where a correlation between MR-visible lipids, tumor malignancy and necrosis was observed in brain tumors. These initial findings were subsequently confirmed in *in vivo* imaging studies as well as in histologically different brain tumor types (131). An early demonstration of the sensitivity of lipid signals for accurate diagnosis of supra-tentorial brain tumors was demonstrated by Preul *et al.* (132) suggesting lipid signals as a potential biomarker for grading of astrocytomas. Correlation of lipids with malignancy was also reported in pediatric brain tumors (133) and a further study in pediatric brain tumors suggested the presence of lipids as a marker of poor survival (134). Taken together, the presence of MR-visible lipid signals seems to be a marker for malignancy and poor prognosis. In our recent review on the role of lipids in tumors (92) we argued that increased MR-visible lipids in tumors are indicative of a stress response and that their localization reflects areas of cellular hypoxia and or necrosis.

MRS methods have been used to assess changes in the metabolic profile of tumors as markers of early therapeutic response. Most xenograft and clinical studies have focused on evaluating reductions in the total choline or lactate signal as a marker of treatment response. Changes in lipid signals not only suggest a positive response, but increases in PUFA, a putative marker of apoptosis (106), may also aid in understanding the mechanism of cell death induced by specific treatments.

Gene therapy induced changes in lipids

One of the first studies to implement MRS to evaluate changes in metabolism during gene therapy reported a progressive increase in the lipid resonance at 1.3 ppm in 9L rat tumors that were injected intratumorally with adenoviral HSV-*to* followed by ganciclovir treatment (135). These initial studies were followed by several studies of the HSV-*tk* positive BT4C glioma model treated with ganciclovir (106,129,136), which established accumulation of PUFA resonances as a marker of treatment induced apoptosis. The PUFA resonances arise from phospholipase A2 activity leading to hydrolysis of phospholipids in the cell membrane (137). These studies indicate that early increases in mobile lipid resonances after therapeutic intervention may be suggestive of apoptosis and positive treatment response. The temporal evolution of these resonances may be variable, with a decrease in lipid resonances during formation of scar tissue after cell death and lysis. However, the mode and nature of therapeutic interventions could affect changes in lipid resonances. A recent study involving oncolytic viral treatment of immunocompetent Syrian hamster carcinomas as well as a patient with neuroblastoma reported lower unsaturated fatty acids, as measured from the olefinic resonance at 5.3 ppm (Figure 3) in responding tumors than in non-responding tumors (138), which appears to be an exception to the norm. Unlike previous studies, which resulted in an apoptotic tumor cell death after treatment, treatment with oncolytic viruses resulted in multifocal necrosis and a substantial cellular inflammatory response. Taken together, these studies indicate that changes in PUFA resonances could possibly be used to differentiate apoptosis from necrosis, although further data is needed to substantiate this finding.

A decreased PUFA resonance, in comparison to normal breast tissue, was reported in a patient with breast cancer using selective multiple quantum coherence spectroscopy (139). In

contrast to most tumors, a decrease in total tumor lipids was observed in pancreatic cancer compared to pancreatitis as evaluated by MR studies of patient biopsy samples (140) as well as *in vivo* comparing focal pancreatitis to pancreatic adenocarcinoma (141). Although the exact reason for this apparent anomaly is unclear, the pancreatic tumor microenvironment includes stromal tissue that comprises up to 80% of the tumor mass (142). Thus, it is possible that the dominant factor contributing to the MR visible lipid signal in pancreatic cancer arises from the dynamic assortment of extracellular matrix components, infiltrating immune cells, macrophages, pancreatic stellate cells, vascular cells, fibroblasts and myofibroblasts.

Chemotherapy induced changes in lipids

There have been several MRS studies describing alterations in lipid levels in response to treatment with cytotoxic agents. Increased triglyceride resonances were observed in the malignant MDA-MB-435 breast cancer cell line treated with the anti-inflammatory agent indomethacin (143), a drug that has been shown to reduce tumor invasion and inhibit metastasis. Similar increases have also been observed in cells treated with antimetabolic agents and other cytotoxic drugs (96,144-148). Treatment of DU145 prostate cancer cells with the differentiating agents phenylacetate or phenylbutyrate also led to a time dependent increase in lipid signals, accompanied by increased in GPC, indicating cell stress that precedes the induction of apoptosis (107,147). Increased apoptosis in BT4C glioma cells after exposure to cisplatin resulted in increases in both saturated fatty acids and PUFA resonances (149). Treatment with subcytotoxic doses of cisplatin induced similar increases in saturated and total unsaturated fatty acid (5.3 ppm) resonances in HER2+ ovarian cancer cells (150) (Figure 4). Exposure of the VEGFR tyrosine kinase inhibitor SU1498 on human U87 glioma cells induced a significant increase in lipids with a concomitant decrease in GPC (151). Although cell studies point towards

an increase in MR-visible lipid signals in tumors as a marker of response to chemotherapy, translation of these findings to *in vivo* settings have been challenging due to the confounding presence of lipid signals from hypoxic/necrotic regions in the tumor or contamination from lipid signals outside the tumor.

Many factors influence *in vivo* MRS and make it difficult to predict whether it will provide useful biomarkers for new therapeutic interventions. For example, the PI3K/mTOR inhibitor voxali in the presence or absence of temozolomide directly alters the phosphorylation of the phospholipid PtdIns in glioblastoma tumors, but causes no detectable changes in ^1H MR spectra, even though hyperpolarized ^{13}C MRS showed reduced conversion of pyruvate to lactate (152). Nevertheless, many studies have successfully evaluated changes in lipid signals in response to chemotherapy in tumor xenografts. The efficacy of etoposide, a topoisomerase inhibitor, was tested on a murine lymphoma model and a significant increase in the 1.3 ppm methylene mobile lipid resonance was noted following etoposide-induced apoptosis (153). Treatment of F98 rat gliomas with the choline kinase inhibitor MN58b resulted in a significant increase in both saturated (1.3 ppm) and polyunsaturated (2.8 ppm) fatty acids (Figure 5) that correlated with increased treatment-induced apoptosis (114). Similar increases in saturated and polyunsaturated lipid resonances were observed in GL261 glioma tumors in mice treated with temozolomide (128) (Figure 6). In contrast, treatment of C6, RG2, and GL261 glioma models with the nitrones, PBN (α -phenyl-tert-butyl nitron) and OKN007 (2,4-disulfophenyl-PBN) resulted in a decrease in lipid resonances after treatment, which was attributed to a decrease in necrosis and normalization of the metabolic profile rather than the increase in apoptosis generally reported in tumor treatment studies (154). Increases in neutral lipid resonances were also correlated with tumor growth arrest and treatment response in a mouse model of HER2+

ovarian cancer treated with cisplatin (150). No changes in total choline were noted in this study, as the authors found changes in neutral lipids to be a more sensitive marker of treatment response.

Radiation therapy induced changes in lipids

Radiation therapy is one of the most commonly used therapeutic strategies for solid tumors in the clinic. Although several studies point towards increased lipid resonances in radiation-induced necrosis, relatively few studies have evaluated changes in lipid resonances as a marker of early response to radiation therapy. Cervical cancer biopsy samples obtained from patients treated with radiation therapy were studied by HR-MAS, which revealed a direct correlation between the degree of apoptosis and lipid resonances (155) confirming the observations made in cell and xenograft studies. A xenograft model of non-Hodgkin's diffuse large B cell lymphoma treated with radiation therapy demonstrated a significant increase in both mono and unsaturated lipid resonances within 3 days of treatment (156). The increase in lipid resonances corresponded with histology findings of increased apoptosis and oil red O staining, supporting the idea that unsaturated (PUFA) lipid accumulation is a marker of therapy induced apoptosis.

D. Positron Emission Tomography

Positron emission tomography (PET) measures the gamma rays produced when an electron collides with a positron emitted from a beta-decaying nucleus. PET scans require the administration of a radioactive substrate, but the high sensitivity of PET means that only tracer levels of radiolabeled material are needed. For tumor imaging, ^{18}F -fluorodeoxyglucose (FDG) is by far the most commonly employed PET tracer (Figure 7). FDG exploits the enhanced aerobic glycolysis observed in many tumors, a phenomenon known as the Warburg effect.

Overexpression of plasma membrane glucose transporters and hexokinase activity rapidly internalize FDG and phosphorylate it in the first step of glycolysis. This reaction traps FDG-6-P in the cell, but the substrate is unable to be metabolized further. FDG-PET is widely used clinically for the staging of primary tumors, but because of the ability of PET to perform a full-body scan, it is also important for the detection of metastases. FDG-PET can also be used to monitor tumor regression post-therapy, and has been used in several instances to monitor FASN inhibitor therapies due to the link between glycolysis and fatty acid synthesis (157,158). One caveat is that it is difficult to assess enhanced FDG tumor uptake in tissues with high glucose utilization (e.g. brain and heart) or in organs of excretion (e.g. kidney and bladder). There is also the potential for background labeling in metabolically active cells, such as lymphocytes in sites of inflammation. Furthermore, some tumors, such as the prostate, are not FDG avid and have relatively low uptake.

A promising alternative to FDG for tumor lipid imaging is ^{18}F or ^{11}C labeled choline (Figure 7). This strategy relies on the upregulation of choline transporters and ChoK in tumor cells. The concerted action of these components achieves tumor tissue contrast by rapidly internalizing and phosphorylating the choline mimetic radioligands. Choline PET is becoming more widely used for tumor detection, especially in the prostate (159) but also in other tumors such as gliomas (160). Choline PET has also been used to track tumor recurrence, metastasis, and response to chemo and radiotherapy (161-164). One challenge facing choline PET is the discrepancy between labeled choline tracer uptake and steady state metabolites observed using MRS (165). This could be due to intrinsic differences between choline transport and phosphorylation that manifest as differences in tracer uptake relative to steady-state levels, but it

could also be due to alternative pathways such as metabolism of the choline tracers in other organs such as liver (166).

The low FDG avidity of prostate tumors is linked to their metabolic preference for β -oxidation of fatty acids, rather than glutaminolysis or glucose metabolism, as a primary energy source (42). A variety of ^{18}F -labeled fatty acid analogs have been synthesized (Figure 7), and the metabolism of these probes effectively trap the radiolabel specifically in cells with high usage of β -oxidation (167,168). In metabolizable probes, labeling at the hydrophobic end of the molecule allows these probes to be consumed through β -oxidation. In non-metabolizable probes, chemical modifications are nearer to the carboxyl group, which still allows cell uptake and potential incorporation into phospholipids or triglycerides, but further oxidative metabolism is prevented (169). Labeled fatty acid probes, such as BMIPP (Figure 7), have been primarily employed to monitor metabolism in tissues with high levels of β -oxidation, mostly cardiac and to a lesser extent skeletal muscle (170-176). Use of these probes to study cancer has been limited, even though the use of β -oxidation as a potential cancer energy source has been known for decades (177,178).

Although the half-life of ^{11}C is quite short (20 min), it is still possible to observe ^{11}C palmitate uptake and distinguish storage in triglycerides vs metabolism by β -oxidation (179,180). Regions of high fatty acid synthesis have been imaged using ^{11}C -acetate incorporation into palmitate (181), although the intermediate acetyl-CoA has other potential metabolic fates such as the Krebs cycle or histone modification. For this reason, ^{11}C -acetate has been used to monitor prostate tumors during therapy and recurrent lesions, but is incapable of distinguishing malignant tumors from benign hyperplastic nodules (182-185). Several radiolabeled ether lipids containing PC moieties (186) have been made (Figure 7) based upon early observations of elevated

phospholipid-ethers in neoplastic tissue (187,188). Due to the preferential accumulation and retention of these alkylphosphonium analogs, ^{124}I -CLR1404 was developed and recently tested in brain tumor PET imaging where a tumor/normal brain signal ratio of 30 was reported (189). Replacement of the PET isotope with the radio-ablative ^{131}I -CLR1404 agent improved survival in tumor-bearing rodents and provided a novel theranostic platform wherein ^{124}I can assist in the planning of ^{131}I therapy.

E. Mass Spectrometry Imaging

Mass spectrometry imaging (MSI) is a powerful modality for the detailed spatial detection of protein, metabolite and drug distribution in tissues. MSI consists of a family of techniques that includes matrix-assisted laser desorption ionization (MALDI) MSI, secondary ion mass spectrometry (SIMS), matrix electrospray-MSI (ES-MSI), and nanostructure-initiator mass spectrometry (NIMS). For the detection of lipids, the most commonly used technique is MALDI MSI (190), which has a large range of applications and provides a good overall combination of spatial resolution, intact molecule sensitivity and probing depth (190). MALDI provides a critical platform for the spatial detection of lipids and lipid metabolites, as there is a lack of suitable reagents and antibodies for the analysis of tissue lipids *in situ* (191).

MSI techniques are invasive procedures, requiring the preparation and embedding of a thin tissue slice followed by rasterized destruction by laser or primary ion beams. For MALDI, tissue is embedded in a matrix such as gelatin and α -cyano-4-hydroxycinnamic acid (CHCA) or 2,5-dihydroxybenzoic acid (DHB) (192). Washing the tissues with aqueous solutions of ammonium formate or phospholipases prior to embedding can be used to enhance certain lipid species and improve signal to noise (191). MSI can detect hundreds of molecules in a single sample with a routine spatial resolution of 50–100 μm , and a small molecule resolution as high

as 5–10 μm (193). Because matrix deposition is a slow process and the application of a wet matrix to tissue can cause metabolite shift, matrix-free techniques using nanoparticle initiators have been developed and these often provide simpler spectra to interpret (194,195). These NIMS procedures provide comparable lipid profiles, but with improved spatial resolution (196).

MSI has been used extensively to study lipid in primary human tumor tissue as well as tumor cell lines and xenografts. In human tissues, a consistent observation of elevated phospholipids has been observed in cancer compared to normal tissues. This includes breast tumors and invasive ductal carcinomas compared to carcinoma *in situ* (197-199), gastric cancers (200), renal cell carcinomas (201), human lung squamous cell carcinoma (202) and colorectal carcinoma (203,204). The most frequent observation is of increased PtdCho in tumors, but increases in PtdEtn, PtdIns, and PA have also been observed (199,205,206). Some studies have demonstrated increases in ether-linked lipids (199). Lyso-phospholipids have been reported to increase in colorectal tumors, but decrease in gastric and prostate cancers (200,203,207) (Figure 8). Fatty acid levels have also been observed to increase in a number of tumors (199), but it is uncertain whether these are free fatty acids or acyl components of more complex lipids.

Studies in tumor xenografts have confirmed the general observations of increased phospholipids and ether-linked lipids in tumor tissues. Measurement of choline metabolites in breast cancer xenografts using MR spectroscopic imaging combined with MALDI-MSI revealed differences in spatial distribution with PC and choline levels concentrated in normoxic tumor regions (208). When correlated with the expression of a hypoxia-response element, PtdCho elevation was concentrated in hypoxic regions whereas LPC was elevated in necrotic regions (192,209). Further studies have indicated distinct microenvironmental lipid distribution with higher levels of ether-linked PtdEtn in viable tumor and ether-linked PtdCho in necrotic regions

(193). Elongated fatty acyl chain length in phospholipids of human lung squamous cell carcinomas was reproduced in mouse xenografts and correlated with the expression of the acyl chain elongase ELOVL6 (210). These data demonstrate the role of MSI in identifying distinct tumor microenvironments by their molecular signatures.

While MSI has revealed a number of potential lipid-related cancer markers, there are very few studies that have employed MSI lipids to monitor cancer treatment. One recent study has identified transient decreases in serum PtdCho and lyso-PtdCho associated with response to radiation in head and neck tumor patients (211). A second study has indicated that LPC (16:0) is a significant predictor of PSA recurrence in human prostate cancer patients (207). The NIMS technique is capable of simultaneous monitoring of drug accumulation and endogenous water-soluble metabolite distribution (212,213). Adopting this technique to study drugs that target lipid metabolism in tumors should be a priority in this field. These data indicate that further investigation into the utility of lipid biomarkers to assess treatment response is warranted.

F. Optical Imaging

Optical imaging is a cost-effective and relatively easy-to-use imaging modality that makes it a valuable tool for non-invasive longitudinal imaging. The range of detectable wavelengths is broad enough to allow multiplex imaging, and these principles have been applied to a number of medical diagnostic platforms, including flow cytometry, fluorescence microscopy, and diffuse optical imaging. *In vivo*, optical imaging is limited by attenuated light penetration through tissue due to scattering and absorption in the visible wavelengths. This is partially alleviated by using chromophores that absorb or emit in the near infrared region (675 – 900 nm) where absorption by hemoglobin, deoxyhemoglobin and water is at a minimum. In

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3 addition, several endogenous proteins, such as laminin and elastin, autofluoresce and elevate the
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5 background signal during *in vivo* measurements. As a result, the observable depths of optical
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7 imaging are usually limited to 1 cm or less. Thus *in vivo* optical imaging has predominantly
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9 focused on single-channel fluorescence or bioluminescence on a macro level (< 15x
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11 magnification) in mice or other small animals. Bioluminescence is not practical in clinical
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13 settings, because it requires transfection with an invertebrate luminescent protein, but it is a
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15 highly sensitive reporter that, in the field of lipid metabolism, has been adapted to map regions of
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17 heightened fatty acid uptake (214). Advances in CCD sensitivity has recently sparked renewed
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19 interest in Cerenkov radiation as an additional source of emitted light that can be detected *in vivo*
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21 (215,216). This signal may one day offer functional information concordant with the
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23 tomographical information provided by PET (217).
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29 New optical imaging technologies in oncology have largely followed advancements in
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31 vascular imaging. Before diffuse optical tomography became a subject of interest in
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33 mammographic screening, the observation was made that absorption of circulating hemoglobin
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35 changes in an oxygen-dependent manner (218). This was a pioneering study in translational
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37 optical imaging, but 20 years later routine clinical optical imaging remains limited outside of
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39 optical coherence tomography (OCT) in ophthalmic applications (219-221). OCT uses a rastered
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41 application of NIR light and interferometry to produce a 3-D scanning image of the retina. The
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43 use of NIR wavelengths still limits the application to surface phenomena, less than 1 cm deep in
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45 the tissue. OCT has also been employed in oncology, most notably to detect the development of
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47 esophageal tumors (222,223). With regards to lipids, OCT is most commonly used for
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49 atherosclerotic plaque detection in cadavers and patients, which is based upon its sensitivity to
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lipid deposits in local vascular sites (224,225). This leads to the possibility for future applications of OCT as a method to distinguish lipid levels in treated tumor tissue.

In small animal optical imaging, enhanced contrast is often provided by the administration of exogenous NIR fluorescent compounds. There are a growing number of these molecular probes that allow for noninvasive optical imaging of lipid metabolism pathways. The probes are generally of three types: passive molecules that are used as blood pool agents and rely on leaky vasculature to accumulate at the site of pathology, targeted permanently fluorescent molecules that can be taken up selectively as substrates or inhibitors of lipid metabolic pathways, and smart quenched fluorescent molecules that can be selectively activated by enzymes. Fluorescence imaging using these probes allows for specific and localized detection of metabolic activity.

Indocyanine green (ICG) is a clinically approved blood-pooling agent that has been used for tumor detection. Collection of ICG in solid tumors has been attributed to the enhanced permeability and retention caused by disordered vasculature and insufficient lymphatic systems in tumors (226), however tumor-associated macrophages in lipid-rich regions may have an underappreciated role in this phenomenon. In atherosclerotic plaques, ICG has been observed to accumulate preferentially in the lipid-rich macrophages in both rabbits and patients (227). MRI, PET, and optical imaging of tumor-associated macrophages have been described extensively (228-230) and these methods should be used to explore co-localization of lipid profiles with immune infiltration in the tumor setting.

Lipid uptake and synthesis

Lipid uptake and synthesis are essential to the maintenance of mammalian cell membranes. For this reason, a constant source of precursors are required to maintain the

proliferative nature of the cancer cell. Tumor cells synthesize and accumulate membrane lipids, such as PtdCho, at a rate that greatly outpaces the surrounding normal tissue, and this offers an avenue that can be exploited for selective delivery of imaging and therapeutic agents. The introduction of fluorescently labeled lipid analogs into the tumor microenvironment can also be used to visualize lipid scavenger activity. Direct fluorescent labeling of PtdCho has been reported using visible fluorophores, so high background in normal tissues is a general feature of these approaches (231). A common approach is to employ fatty acid probes to which non-polar dyes such as BODIPY have been attached (232). Depending on the metabolic state of the cell, these lipid analogs can be incorporated into phospholipids or neutral lipids in lipid droplets. The alkylphosphocholine analogs described for PET imaging have also been adapted with BODIPY or heptamethine dyes for imaging of tumor margins and draining lymph nodes (233).

Choline kinase

Choline kinase catalyzes the conversion of choline to PC, and is elevated in several types of cancers. Its upregulation has been correlated with the transition of normal breast epithelium toward a malignant phenotype, and selective inhibitors have been developed for Phase I clinical trials in solid tumors (ClinicalTrials.gov, NCT01215864). Based on these studies, we developed a series of fluorescent inhibitors that selectively bind ChoK and emit fluorescence in the near infrared optical window (234). The design exploited the structural similarities between ChoK inhibitors and cyanine dyes frequently employed for optical imaging, and did not rely on dye conjugation to existing inhibitor structures (235). The probe, JAS239, showed elevated uptake in tumors that overexpressed ChoK, and more importantly, had reduced uptake in tumors after chemotherapy (236).

Phosphatidylinositol signaling pathway

Many oncogenic signaling pathways are mediated by PtdIns, which makes these lipids attractive targets for cancer imaging probes. In order to interrogate the role of these phospholipids on cell signaling, Yoon *et al.* engineered a fluorescent PIP₂ sensor to study its role in membrane remodeling, regulation of membrane proteins, and regulation of the cytoskeleton (236-238). This probe was engineered with the visible fluorophore (2-dimethyl-amino-6-acyl-naphthaline) optimized for its lipophilic properties, limiting its utility for detection of lipid dynamics *in vivo*. Because IP₃ and DAG mediate the release of intracellular calcium and are activated by lipid metabolic pathways, optical probes based upon flu-4 or BAPTA can provide an indirect means of assessing these bioactive lipids (239-241). DAG can be evaluated directly using radiolabeling (242) or using optical techniques such as FRET to detect activated DAG intercalation in the plasma membrane (243).

Phospholipases

Phospholipases are catabolic enzymes that can be targeted with lipid based quenched optical probes that are activated by enzymatic hydrolysis (39). Upregulation of cPLA₂ has been observed in breast and prostate cancers (112), and elevated levels of PC and phosphoethanolamine have been attributed to PLC activation in breast cancer, ovarian cancer and melanoma (109). Activatable probes with phospholipid-based structures, a linker that regulates enzyme accessibility, a quenchable fluorophore or light-emitting compound, and a cleavable quenching domain have been designed as fluorescent agents for quantifying phospholipase activity. The advantage of this approach is the enhanced signal to noise available from the continuous enzymatic release of fluorescent substrates. Enzyme activated BODIPY probes have been employed to examine phospholipase activity in many systems including the

digestive physiology of the zebra fish (244-247). While useful for cells, small transparent animals and intravital microscopy, the optical properties of BODIPY limit its detection in tissue. There are several quenched fluorophores commercially available with emission in the visible range. In the NIR range, we developed analogous probes targeting PLA₂ and PLC for *in vivo* applications (248). These self-quenching probes were composed of pyropheophorbide-*a* (Pyro) tethered to PtdEtn and a Black Hole Quencher (BHQ-3). While Pyro has an excitation maximum ~~excitable-Soret band~~ at 418 nm that emits at 660 nm, its absorption peak at 670 with emission at 725 nm makes it suitable for *in vivo* imaging. The specificity of these probes to various phospholipase isoforms could be modulated by altering the spacer length between Pyro and the glycerol backbone. The resulting construct, Pyro-PtdEtn-BHQ, was highly specific to PtdCho-specific PLC (PC-PLC) both *in vitro* and *in vivo* and we used this to detected upregulation and activation of this enzyme in DU145 prostate cancer xenografts (Figure 9) (112).

Arachidonic acid, a PUFA found in the *sn*-2 position of phospholipids, is mobilized by the activity of cytosolic or cPLA₂ acting on membrane-associated phospholipids. The redox activity of COX-2 catalyzes the conversion of arachidonic acid to prostaglandins that act as a lipid second messenger. Arachidonic acid is first converted to prostaglandin G₂, and then the peroxidase activity of COX-2 converts it to the unstable H₂ isoform that is converted to one of several homologous tissue- and function-specific isoforms of prostaglandins. It is prostaglandin E₂ that is notably upregulated in several types of cancer contributing to aberrant signaling (249). In order to utilize this signal transduction pathway to image pathogenic events, there are several points of interrogation possible: using activatable probes to evaluate COX-2 activity, directly measure PGE₂ levels with targeted probes, or evaluate activation of further downstream events.

Detection of cPLA2 using a cleavable construct requires a probe that contains arachidonic acid, to account for the specificity of the enzyme for this fatty acid, as well as a small enough head group to fit into the active site. Previous studies had shown that arachidonic acid esterified to visible fluorophores such as 7-hydroxycoumarin were highly specific for cPLA2 and displayed similar hydrolysis kinetics to native substrates (250). We adapted this caged fluorescence protocol using fluorophores that emit in the red wavelength range (660-680 nm) to create cPLA2 sensitive probes suitable for *in vivo* imaging. Probe selectivity could be modulated by choice of fatty acids and fluorophores, with DDAO arachidonate performing the best *in vitro*, in cells and tumors. Here, arachidonic acid provided the optimum fatty acid substrate for cPLA2 and DDAO (7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one) was the most resistant to non-specific aqueous hydrolysis. Probe activation was shown to be proportional to cPLA2 expression levels in cells and tumors, and high relative to a non-specific control probe, DDAO palmitate.

Apoptosis

In addition to using imaging techniques to noninvasively detect components of lipid metabolism, other biomarkers can be detected that offer information pertaining to non-homeostatic states of cells and tissues *in situ*. Apoptosis is often indicative of patient response to cancer therapy, accordingly, targeting markers of apoptosis can be useful to evaluate the efficacy of various interventions (251). Among these events are plasma membrane reorganization marked by PtdSer translocation to the outer leaflet of the membrane. PtdSer translocation can be detected using labeled ligands that bind anionic phospholipids, including proteins such as Annexin 5 or synaptotagmin (252) or organic molecules such as zinc dipicolylamine (253). A number of

labeling strategies have used this approach to create apoptosis sensors for optical (254), MR (255-257) and nuclear imaging when radiolabeled with ^{99}Tc or ^{123}I (258). Annexin has shown some promise as a molecular probe for this purpose though this strategy can be limited by suboptimal pharmacokinetics (259), and non-specific labeling of necrotic cells (260).

Conclusion

Lipid metabolism is an essential component of cellular homeostasis that can become drastically altered during the process of malignant transformation. Most common driver mutations and oncogenes are related to growth cues and cellular stress responses, and many therapeutic strategies target these pathways such that the measurement of lipid metabolic changes can serve as a proxy for drug response pharmacodynamics. While MRS and PET approaches offer the ability to serially-measure lipid composition and flux, they are limited by spatial resolution. MSI and Optical Imaging, on the other hand, permit the appreciation of intratumoral heterogeneity but often require surgical exposure of the tissue site. Still, these approaches are finding increasing value in image-guided surgical resection and are experiencing fast-paced improvements in technology including the ability to image endogenous contrast, more specific probes that provide kinetic information, and sub-cellular resolution. For a balance between resolution and ability to image endogenous lipid signals without the need for new FDA-approved contrast agents, MRS remains the gold standard for measuring lipid metabolic states in tumor and other tissues. New advances including two-dimensional MRS, hyperpolarized MR spectroscopic imaging, and higher field strength MRS continue to reshape our understanding of lipid metabolic changes during tumor growth and treatment response. This comes at a critical time as therapy continues to evolve with new signal transduction inhibitors, metabolic targeting agents, and immunotherapies change the landscape of cancer care.

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Figure Legends

Figure 1: Lipid metabolism pathways in the cell. Glucose transported into the cell is metabolized to pyruvate via the glycolytic pathway, and can be fed into the tricarboxylic acid (TCA) cycle for ATP production in the mitochondria. Citrate from the TCA cycle or from exogenous sources is converted to Acetyl-CoA in the cytoplasm and can be used for cholesterol or fatty acid (e.g. palmitate) biosynthesis. Cholesterol produced in this fashion can be used to synthesize membranes or be incorporated into sterols. Fatty acids can be desaturated or extended and then incorporated into triglycerides or incorporated into phospholipids via the Kennedy Pathway. Cholesterol and triglycerides fatty acids can also be taken up through specific transport mechanisms. The resulting lipid droplets, shown in the inset, contain fatty acids stored in phospholipid and fatty acid form that can be mobilized by phospholipases and lipases, respectively. Beta-oxidation (β -Ox) of free fatty acids from lipid droplets can be triggered to meet energy needs. In place of the third acyl group (Acyl_3) attached to the glycerol moiety of triacylglycerides (orange oval), phospholipids have a phosphorylated polar head group (yellow sphere) that confers water solubility and creates organized bi-layer and micelle structures. Phospholipases can also elicit 2nd messenger signaling cascades by releasing bioactive fatty acids (e.g. sphingolipids, eicosanoids) and/or polar head groups in response to growth or stress cues. External cell signaling cues can also be transmitted by lipids in the case of isoprenylation and subsequent transport of small GTPases to dock with G protein-coupled receptors (GPCRs) on the cell surface.

Figure 2: Major lipid metabolic pathways altered in cancer cells. Metabolic reprogramming during malignant transformation mimics autonomous growth signaling in unicellular organisms. Common oncogenes in the PI3K/Akt/MTOR pathway, are often mutated

or upregulated due to their presence downstream of external growth factors and their ability to re-route cellular carbon sources toward fatty acid utilization as an energy source. Cancer cells can also supply the TCA cycle from glucose via aberrant glycolysis and from glutamine via IDH mutations, although the contributions of these pathways differ greatly depending on the energy needs of the cell. The transcription factor Myc promotes many malignant processes in cancer cells because it is a master regulator capable of triggering survival pathways in response to hypoxia and nutrient deprivation. Hypoxic conditions are a factor in many solid tumors and influence the local tumor microenvironment by dampening the immune response and activating hypoxia-inducible factor-1 α (HIF1 α), which among other things, initiates the regulatory function of sterol regulatory element binding protein 1 (SREBP-1) on fatty acid synthesis. Free fatty acids such as palmitate are used as energy sources or converted to bioactive eicosanoids to dampen the immune response and stabilize the ER stress that can result from saturated fatty acid accumulation.

Figure 3: Changes in lipid metabolism in response to oncolytic viral treatment of Syrian hamster carcinomas. The oncolytic viral treatment induces coagulative necrosis, which is seen as hypo-intense areas within the tumor on T_2 -weighted MR image. *In vivo* MRS from the voxel (overlaid on the image) is shown on the right demonstrating resonances from unsaturated fatty acids, taurine and choline. No changes in PUFA resonances were observed in coagulative necrosis resulting from oncolytic viral treatment. Reprinted with permission from (138).

Figure 4. A) Confocal micrographs of SKOV3.ip cells treated for 48 h with cisplatin (CDDP) show increased lipid droplets as measured by Nile red staining, compared to untreated

controls (CTRL). Fixed cells were counterstained with phalloidin 488 for actin (green) and DAPI for nuclei (blue). Scale bar represents 23.8 μm . (B) ^1H NMR spectra of intact SKOV3.ip cells treated with 5 mM CDDP for 48 h (red) show increases in mobile lipids compared with untreated control cells (black). Labeled lipid resonances include: methine protons at 5.3 ppm ($-\text{CH}=\text{CH}-$); fatty chain methylene group at 1.3 ppm ($-(\text{CH}_2)_n-$); methyl group at 0.9 ppm ($-\text{CH}_3$); and total choline (tCho) at 3.2 ppm ($-\text{N}(\text{CH}_3)_3$). Reprinted with permission from (150).

Figure 5: Increased lipids in response to choline kinase inhibition as an alternate therapy for the treatment of gliomas. *In vivo* MRS from an untreated F98 rat glioma (bottom spectrum) and after 5 days of treatment with a choline kinase inhibitor, MN58b (top spectrum). Increased mono-unsaturated lipid peaks (1.3 ppm) are evident with treatment. In addition, a significant increase in the poly-unsaturated fatty acids (PUFA, 2.8 ppm), indicating apoptotic cell death. As expected, a decrease in the total choline (tCho) peak was also observed in response to choline kinase inhibition. The MR image demonstrates placement of the voxel for MRS studies. Reprinted with permission from (114).

Figure 6: Single voxel ^1H MR spectra from a GL261 tumor implanted into a mouse brain. Treatment with three cycles of temozolomide led to significant (*) increases in mobile lipid:Cre and tCho:Cre resonances at 28 days post inoculation (left) compared to spectra acquired before treatment (right). Labeled resonances: Cho (choline: 3.2 ppm, Cre (creatine): 3.3 ppm, MLs (mobile lipid methylenes): 1.3 ppm, PUFAs: 2.8 ppm. Reprinted with permission from (128).

Figure 7: Chemical structure of the common PET tracers used for detection of lipid metabolism. The radioactive isotope is denoted in red.

Figure 8: MALDI-MSI images and corresponding H&E sections from human colorectal tumors (A-C) and adjacent tumor free regions (D-F). A selective projection of m/z 478.3 onto MALDI-MSI images reveals elevations in 1-palmitoyl-lysophosphatidylcholine in cancer-containing areas compared to the non-tumor bearing tissue sections. Elevations in m/z 504.3, representing 1-oleoyl-lysophosphatidylcholine, were also observed in this study. Reprinted with permission from (203).

Figure 9: *In vivo* NIR fluorescence imaging of phospholipase activity using the PC-PLC activatable probe Pyro-PL-BHQ in DU145 prostate tumor xenografts. Each mouse received 80 nmol *i.v.* of (A) Pyro-PtdEtn, a permanently fluorescent analog used as a control, (B) Pyro-PtdEtn-BHQ or (C) Pyro-PtdEtn-BHQ plus pre- and post- injections of the PC-PLC inhibitor D609. The fluorescence intensity from Pyro-PL-BHQ activation peaked at 6-7 h, and was inhibited by D609 treatment. Fluorescence persisted for up to 31 h in tumor tissue (T) excised from mice when compared to muscle (M) control. Reprinted with permission from (112).

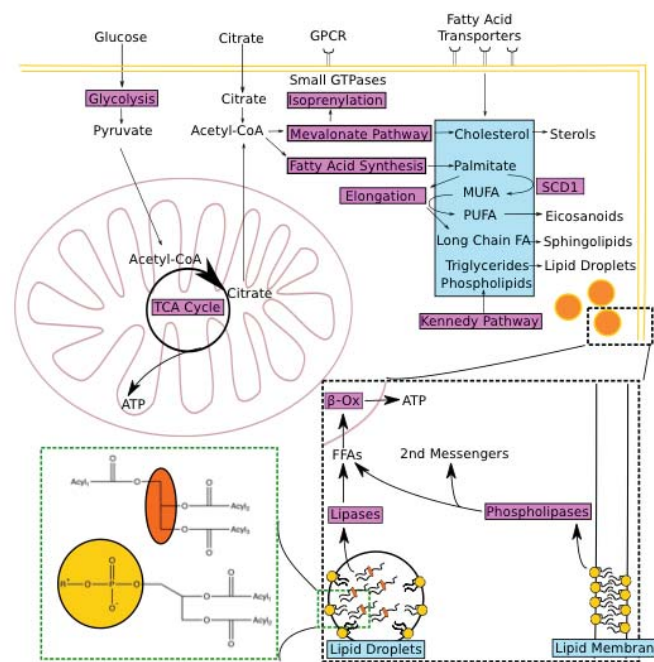


Figure 1

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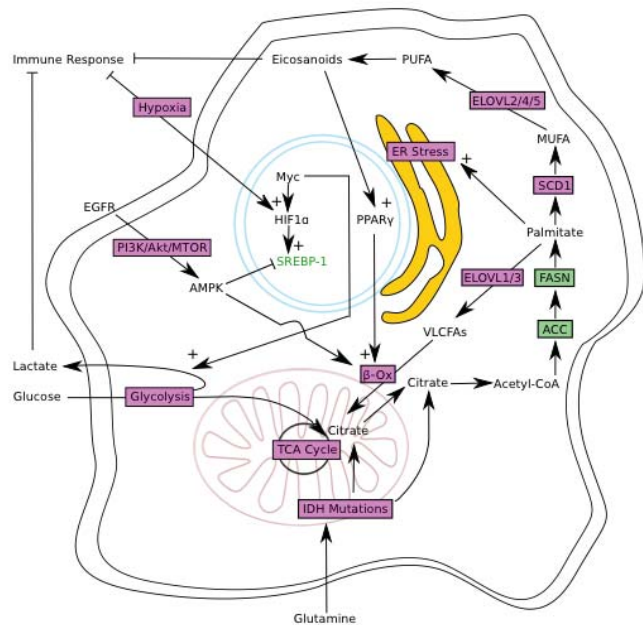


Figure 2

Figure 2: Major lipid metabolic pathways altered in cancer cells. Metabolic reprogramming during malignant transformation mimics autonomous growth signaling in unicellular organisms. Common oncogenes in the PI3K/Akt/MTOR pathway, are often mutated or upregulated due to their presence downstream of external growth factors and their ability to re-route cellular carbon sources toward fatty acid utilization as an energy source. Cancer cells can also supply the TCA cycle from glucose via aberrant glycolysis and from glutamine via IDH mutations, although the contributions of these pathways differ greatly depending on the energy needs of the cell. The transcription factor Myc promotes many malignant processes in cancer cells because it is a master regulator capable of triggering survival pathways in response to hypoxia and nutrient deprivation. Hypoxic conditions are a factor in many solid tumors and influence the local tumor microenvironment by dampening the immune response and activating hypoxia-inducible factor-1α (HIF1α), which among other things, initiates the regulatory function of sterol regulatory element binding protein 1 (SREBP-1) on fatty acid synthesis. Free fatty acids such as palmitate are used as energy sources or converted to bioactive eicosanoids to dampen the immune response and stabilize the ER stress that can result from saturated fatty acid accumulation.

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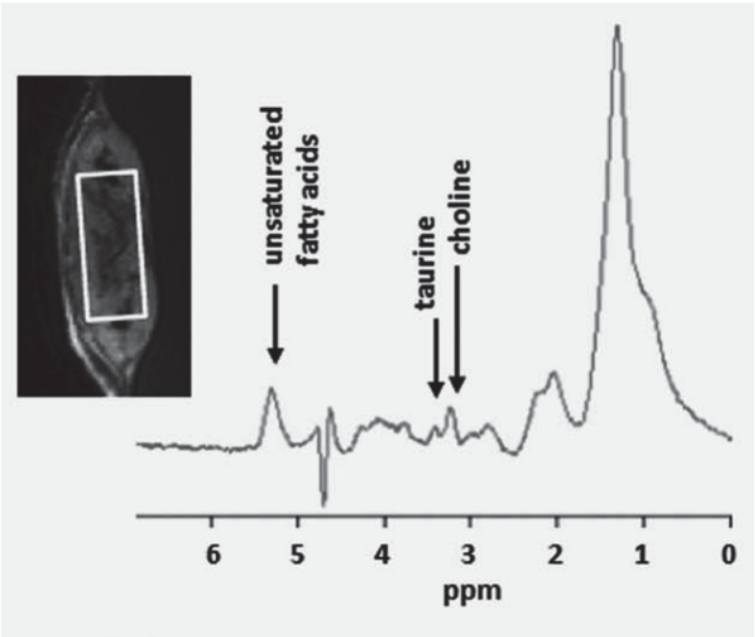


Figure 3

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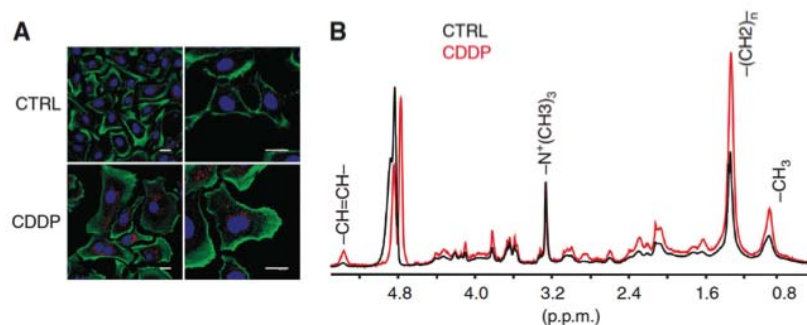


Figure 4

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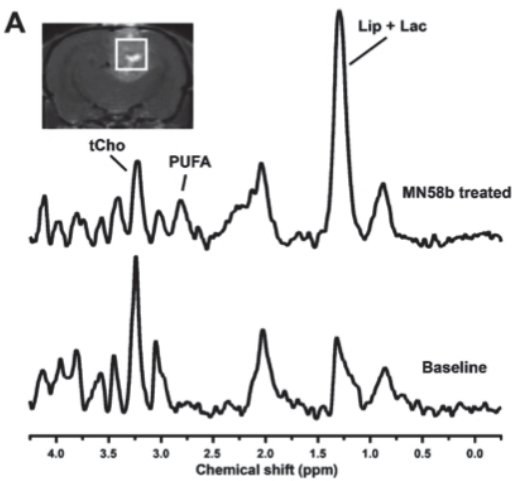


Figure 5

Figure 5: Increased lipids in response to choline kinase inhibition as an alternate therapy for the treatment of gliomas. In vivo MRS from an untreated F98 rat glioma (bottom spectrum) and after 5 days of treatment with a choline kinase inhibitor, MN58b (top spectrum). Increased mono-unsaturated lipid peaks (1.3 ppm) are evident with treatment. In addition, a significant increase in the poly-unsaturated fatty acids (PUFA, 2.8 ppm), indicating apoptotic cell death. As expected, a decrease in the total choline (tCho) peak was also observed in response to choline kinase inhibition. The MR image demonstrates placement of the voxel for MRS studies. Reprinted with permission from (114).

279x209mm (72 x 72 DPI)

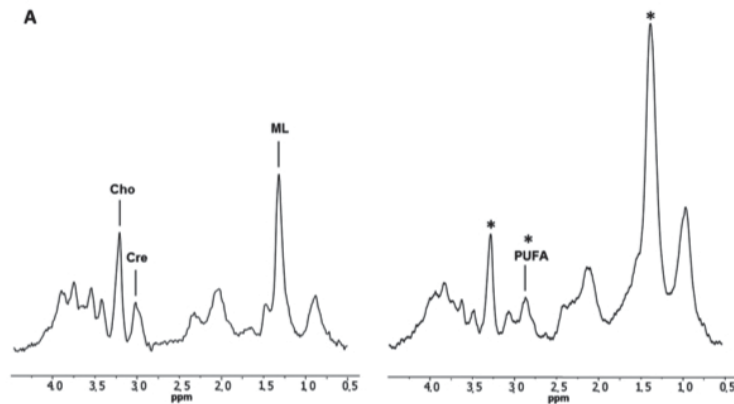


Figure 6

Figure 6: Single voxel ^1H MR spectra from a GL261 tumor implanted into a mouse brain. Treatment with three cycles of temozolomide led to significant (*) increases in mobile lipid:Cre and tCho:Cre resonances at 28 days post inoculation (left) compared to spectra acquired before treatment (right). Labeled resonances: Cho (choline: 3.2 ppm, Cre (creatine): 3.3 ppm, MLs (mobile lipid methylenes): 1.3 ppm, PUFAs: 2.8 ppm. Reprinted with permission from (128).

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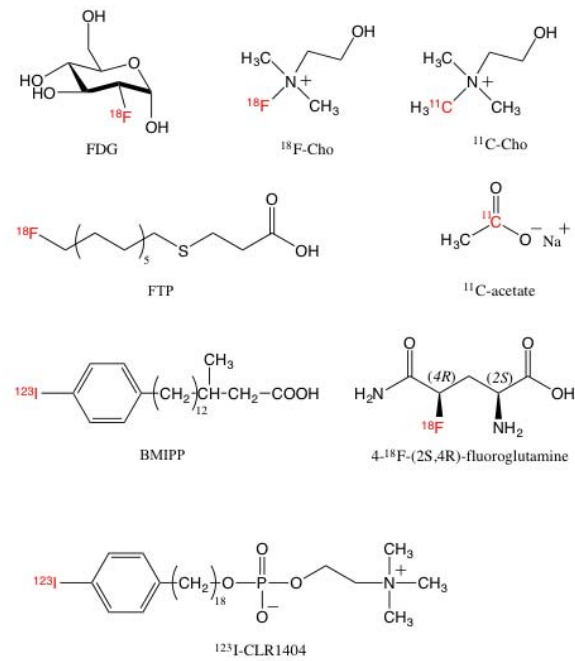


Figure 7

Figure 7: Chemical structure of the common PET tracers used for detection of lipid metabolism. The radioactive isotope is denoted in red.

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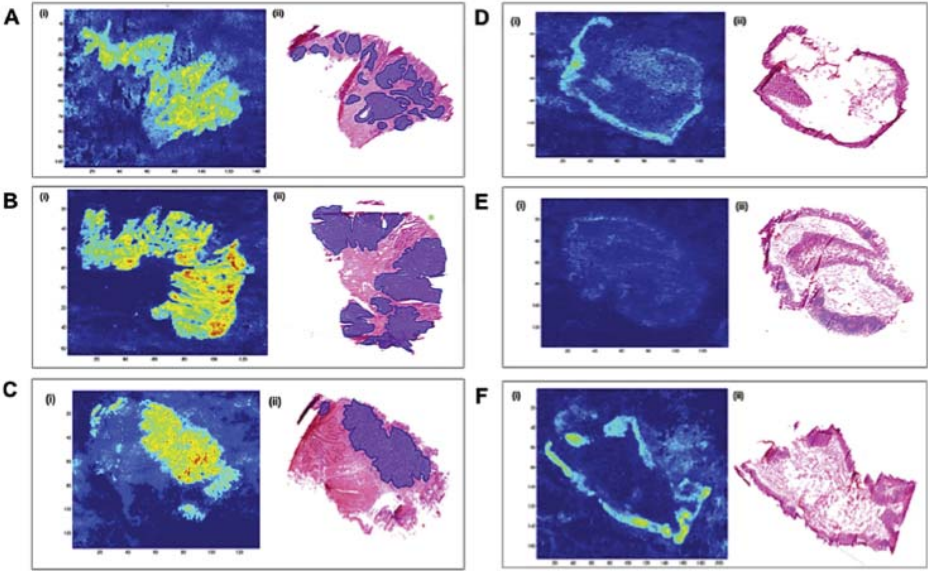


Figure 8

Figure 8: MALDI-MSI images and corresponding H&E sections from human colorectal tumors (A-C) and adjacent tumor free regions (D-F). A selective projection of m/z 478.3 onto MALDI-MSI images reveals elevations in 1-palmitoyl-lysophosphatidylcholine in cancer-containing areas compared to the non-tumor bearing tissue sections. Elevations in m/z 504.3, representing 1-oleoyl-lysophosphatidylcholine, were also observed in this study. Reprinted with permission from (203).

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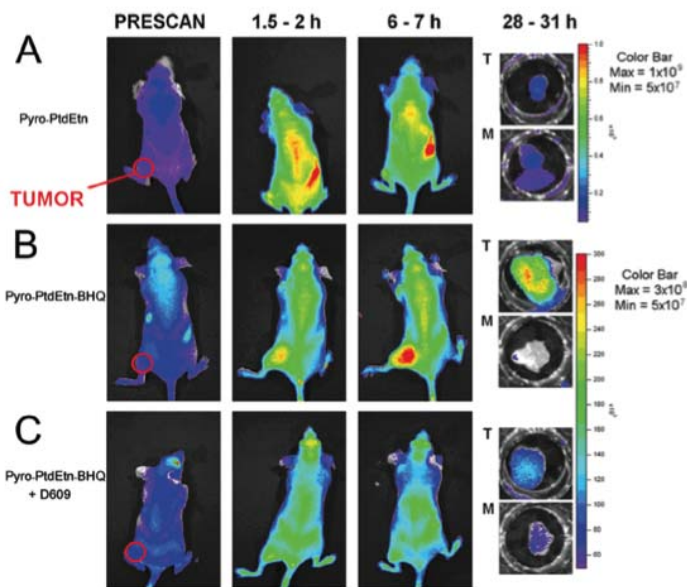


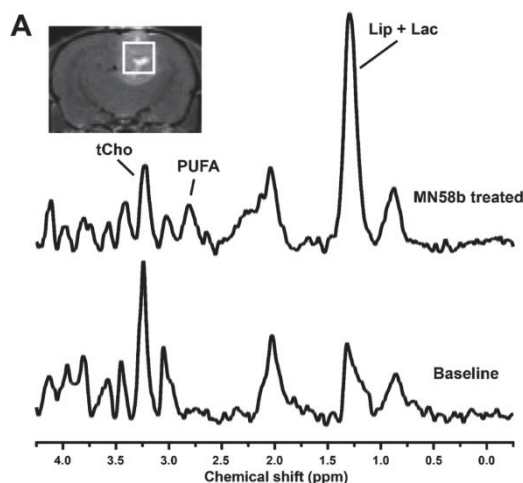
Figure 9

Figure 9: In vivo NIR fluorescence imaging of phospholipase activity using the PC-PLC activatable probe Pyro-PL-BHQ in DU145 prostate tumor xenografts. Each mouse received 80 nmol i.v. of (A) Pyro-PtdEtn, a permanently fluorescent analog used as a control, (B) Pyro-PtdEtn-BHQ or (C) Pyro-PtdEtn-BHQ plus pre- and post- injections of the PC-PLC inhibitor D609. The fluorescence intensity from Pyro-PL-BHQ activation peaked at 6-7 h, and was inhibited by D609 treatment. Fluorescence persisted for up to 31 h in tumor tissue (T) excised from mice when compared to muscle (M) control. Reprinted with permission from (112).

279x209mm (72 x 72 DPI)

Graphical Abstract: Imaging of Cancer Lipid Metabolism in Response to Therapy

Sean Arlauckas, Elizabeth A. Browning, Harish Poptani, E. James Delikatny



Abstract

Lipids play a critical role in biological systems ranging from structural integrity to signaling, energy, defense and communication. This article reviews lipids and lipid metabolic pathways altered in cancer development and their changes in response to therapy that are amenable for study by imaging. We focus first on MR spectroscopy, which was instrumental in defining the field of lipid imaging (Figure) and still plays a major role, followed by complementary molecular imaging methods including PET, mass spectroscopic imaging and optical imaging.