Lipid transactions in cancer—the fat addiction and glycerol-3-phosphate acyltransferase action

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Metabolomics in cancer—what lipids have got to do?

Cancer is a complex-multistep process comprising genetic, molecular and metabolic events. Cancer cells are highly proliferative and invasive, always associated with an upregulation in their "natural building blocks"—nucleic acids, proteins and lipids. Due to a high genetic burden and also because of increasing structural and functional demands, a perturbation arises in the levels of numerous metabolites in biochemical pathways related to these building blocks. The metabolic signatures in cancer have been documented to reach diagnostic accuracy, serving as potential biomarkers. Metabolomics profile was explored in a wide range of cancers so far, including ovarian cancer, oesophago-gastric cancer, colorectal cancer etc, and dysregulation was noted in the pathways of cellular respiration, carbohydrate, protein, nucleotide and lipid metabolism (1). Metabolome shift is pivotal to cancer progression and among the key metabolic pathways, glucose and glutamine metabolism were widely highlighted, and phospholipid-choline metabolism are among the recently dissected candidates. The most popular metabolic change in cancer is the "Warburg effect", characterized by heightened anaerobic glycolysis (lactic acidosis), where ATP generation is significantly obliterated. "Warburg effect" was one of the preliminary observations in cancer, but years later deviation was reported in several other pathways.

Lipids were previously considered only as passive cell membrane components. Pertaining to the lipid metabolism, common alterations in cancer include enhanced fatty acid oxidation, abnormal levels of glycerolipids, sphingolipids and free fatty acids (FA) etc (1). This metabolic rewiring is particularly because of microenvironment stimuli like hypoxia and starvation notable in cancer. In normal healthy cells, FA have multifaceted fates, which encompass: energy storage [triacylglycerols (TAGs), lipid droplets], membrane formation [glycerophospholipids, cardiolipins (CLs) and sphingolipids], generation of signaling molecules [lysophosphatidic acid (LPA), phosphatidic acid (PA), eicosanoids] or oxidized to generate ATP, serving as fuel (2). The cellular FA pool is formed from, exogenous FA arising from blood stream, carried into cells via membrane FA binding proteins (CD36), FA transport proteins or FA translocase or through passive entry; "mono and polyunsaturated FA, cholesterol" can form CLs, sphingomyelins, TAGs, phosphoglycerols, prostaglandins and thromboxanes. FA in cancer is routed through exogenous and endogenous source (2). To support cellular proliferation and biosynthetic activities in cancer cell, an accelerated lipid metabolism is critical (3). In 1953, Medes *et al.* were the first to demonstrate lipid synthesis in tumor tissues (2) (*Figure 1*). Lipids metabolism is dominant in cancer, as FA are essential for the synthesis of cell membranes and signaling molecules, and evidence suggests that cell proliferation can be suppressed through reduction of FA availability to the cancerous cell. Moreover, cells with significant cancer potential like cancer stem cells and aggressive cancer cells were shown to have more lipid content, and elevated anabolic (lipogenic) and catabolic (lipolytic) switching. FA metabolism is a potential metabolic program in cancer, but for a long time was a
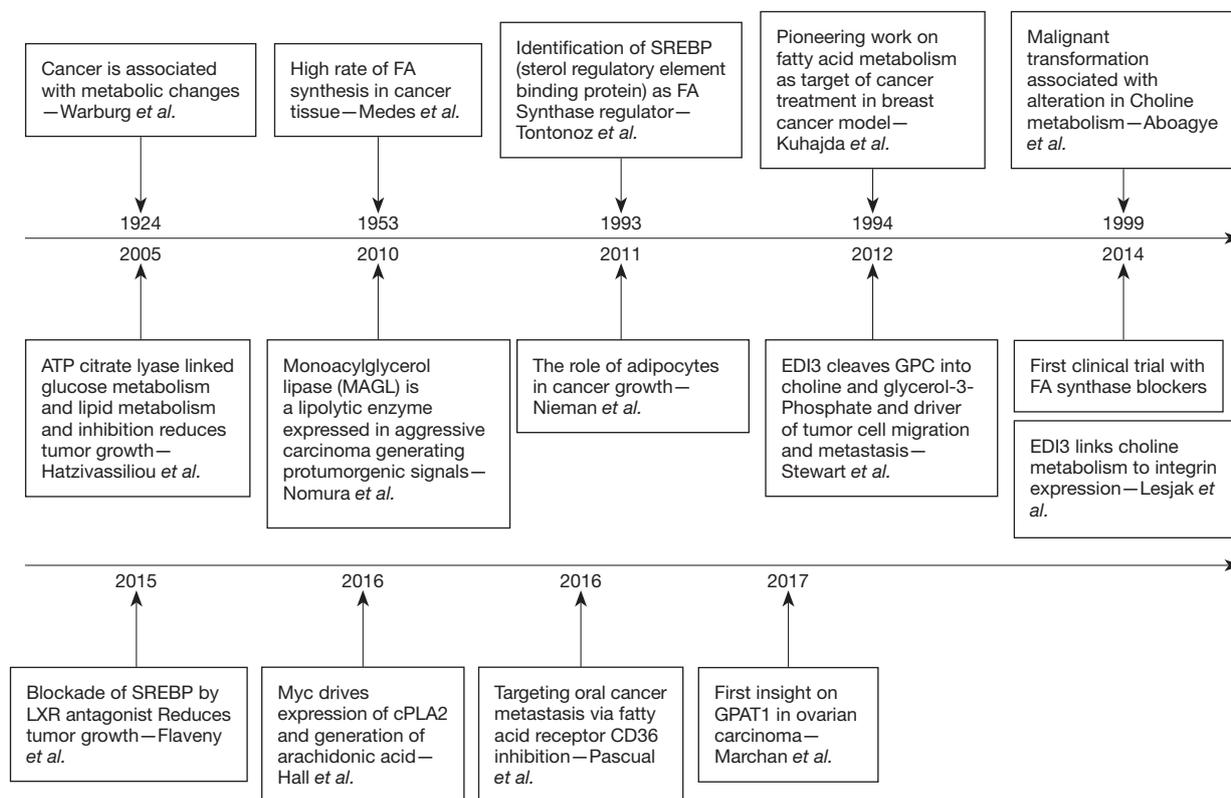
Figure 1 Landmarks in cancer metabolism and pivotal studies in phospholipid-choline metabolism in cancer are shown. The first insight on cancer as a metabolic event began with the discovery of "Warburg effect" by Sir Otto Warburg. Thirty years later, excessive lipid synthesis was identified in cancer. Ever since, the critical steps in phospholipid and choline synthesis were revealed, and some enzymes and their products were shown to have high potential for cancer and targeted inhibition in cancer therapeutics.

missing target for metastatic cancer treatment. Recently several reports, including the extensive studies of Rosemarie Marchan (RM) group, have documented the critical role of lipids in cancer survival and progression, from membrane building and signaling perspective. Among the six hallmarks of cancer (4), the contribution of lipids is mainly towards limitless proliferation and invasiveness–metastasis (3). For the modulation of lipid synthesis glycerol-3-phosphate acyltransferase (GPAT) is an effective target.

GPATs—their mundane roles in lipid metabolism

GPAT is composed of four isoforms that are critical to lipid homeostasis, and their deregulation forms the basis for pathology ranging from obesity and diabetes, to neurological disorders and cancer (5,6). Historically, esterification of glycerol-3-phosphate with a "long-chain acyl-CoA" was shown as the primary step in phospholipids synthesis.

Prospectively, Pullman group suggested that GPAT

comprised of two isoforms, one in outer membrane of mitochondria and other in endoplasmic reticulum. The endoplasmic reticulum (microsomal) activity exhibited no preference for particular acyl-CoA species, whereas the mitochondrial activity preferred saturated acyl-CoAs (e.g., 16:0-CoA and 18:0-CoA) (7). As time passed, four genes were discovered encoding four separate GPAT isoenzymes. The *GPAT1* and *GPAT2* are mitochondrial isoforms, and *GPAT3* and *GPAT4* are endoplasmic reticulum isoforms (7). GPAT1 is resistant to sulfhydryl-modifying reagents like *N*-ethylmaleimide (NEM) inactivation, whereas other GPAT isoforms i.e., GPAT2, GPAT3 and GPAT4 are NEM sensitive (7). Much before, GPAT enzyme system was predicted to contain two set of isoforms, based on their kinetic profile, sensitivity to inhibitors and localization to the sub-cellular mitochondria and microsomes.

GPAT1 is an acyltransferase that specifically drives the "acyl CoA reaction", a rate limiting step in TAG synthesis (7). Its expression is strongest in liver, adipose

tissue and peritoneal pleura which are tissues with dominant TAG synthesis. Furthermore, its expression changes dramatically in starvation and reperfusion. The promoter of *GPAT1* is influenced by carbohydrate, which explains the steady increase in GPAT-mRNA in liver of staved animals refed with carbohydrate diet. As proof of function, the *GPAT1⁻ᐟ⁻* mice showed low weight, less gonadal fat pads, 40% lower hepatic TAG content, 5% lower plasma TAG, and 30% reduced secretion of VLDL-TAG (7). The GPAT1 was also shown to possess defense function; the *knockout* mice had pathology in splenic T cell response to CVB3 antigen of Coxsackie virus (8). *GPAT1⁻ᐟ⁻* mice also demonstrated high mortality, and heart pathology rate (~50%), and cardiac inflammation and oxidative stress, after being subject to CVB3 infection challenge (8). GPAT1 is involved in: acyl-CoA metabolic process, fatty acid-phospholipid homeostasis, response to glucose, LPA-PA biosynthetic process, defense response to viruses, interleukin-2 secretion etc. GPAT2 isoform is essential for normal spermatogenesis (9). The "testis selective" expression of *GPAT2* under physiological conditions was identified from a study that examined 36 different human tissues (10). The endoplasmic reticulum GPAT3 was identified after the characterization of mitochondrial GPAT by Cao and Gimeno group (11). *GPAT3* overexpression results in increased TAG, but not phospholipid formation, so it is a potential lipogenic enzyme (11). GPAT3 was revealed as the primary GPAT of white adipose tissue in *GPAT3⁻ᐟ⁻* mice (11). The knockout mice was alive, and no significant metabolic abnormalities were notable on standard laboratory examinations; they showed decreased body weight gain and increased energy expenditure on intake of high fat food, impairment in cholesterol metabolism, and developed enlarged livers (11). Both endoplasmic reticulum GPAT (i.e., GPAT3 and GPAT4) are phosphorylated at Ser and Thr residues by Insulin, leading to increased GPAT activity, directly linking insulin and "GPAT-3/-4" in glycerolipid biosynthesis.

The knockout and overexpression studies clearly pointed at their putative role in development of hepatic steatosis (excess TAG in liver), insulin resistance, obesity, lactation, and spermatogenesis. In the recent times great emphasis has been put on transcriptional regulation of promoter of *GPAT* by "sterol regulatory element-binding transcription factor 1" (SREBP-1c) regulated by insulin (7). Ideally, optimal orchestration of GPAT system and other lipogenic genes [e.g., FA synthase (FASN)] is necessary for optimal lipogenesis. From animals (12) to humans, GPAT is a potential target to arrest the formation of downstream lipids that support cell membrane formation and downstream signaling molecules like LPA and PA that allow survival and proliferation of rapidly expanding cellular population in cancer (3). The GPAT enzymes have central role in lipid homeostasis and have been indicated in a large number of human cancers.

Linking endometrial differential 3 (EDI3) and downstream GPATs with cancers—how intimate is their relationship?

The role of EDI3 in cancer was first pointed by a team of gynecologists during an attempt to identify metastatic markers in endometrial carcinomas (13). The mechanistic studies were missing at that time, and later investigations confirmed it as a member of glycerophosphodiesterase enzyme family. The substrate of this enzyme and two cleavage products were identified around the same time. The challenge that remained was linking this enzymatic pathway to metastasis. Through lipid analysis Prof. Gerd Schmitz group identified that when EDI3 was manipulated the lipid profile (LPA and PA) altered significantly (13). These lipid mediators were already shown to activate signaling pathways, including migration, adhesion, and proliferation, which are essential to cancer progression. Experiments went on and EDI3 was shown in many models to be consistently linked to cellular migration.

Around 2012, RM group hypothesized that glycerophosphodiesterase EDI3 either directly generates the signaling molecules or provides the membrane anchors for downstream signaling factors (14,15). The EDI3 enzyme was identified to catalyze breakdown of glycerophosphocholine (GPC) to glycerol-3-phosphate and choline (14) (*Figure 2*). Choline is metabolized to phosphatidylcholine (PtdCho), a major lipid in plasma membrane and player in membrane signaling (14). The second product "glycerol 3 phosphate" (G3P) is a precursor to several lipids participating in signaling like LPA, PA, monoacyl-G3P, and diacylglycerol (DAG) (14). LPA has been established as a mitogen and motility factor but its intimate relationship to GPAT was then unknown. It also increases active protein kinase C (PKC) and enhances the migratory ability of tumor cells which signals through six G-proteins coupled receptors (6). Originally EDI3 was hypothesized to induce cell migration through the activation of PKC. In 2014, the same group identified a link between EDI3 and integrin signaling; they showed that EDI3 knockdown in breast and ovarian carcinoma cell line, led to "enrichment of genes in integrin mediated signaling". Knockdown of EDI3 led to reduced expression of "integrin β1 receptor subunit", a key integrin for cell attachment, leading

Figure 2 Deciphered phospholipid-choline pathways. GPC is broken by hydrolysis action of a phosphodiesterase EDI3 to form Cho and G3P. G3P through acylation by GPAT forms LPA. LPA is acted by another acyltransferase (LPAAT) to generate PA. PA-LA conversion is also possible through PLA. PA is an important molecule for membrane lipid biogenesis. PA is converted to CDP-DAG by phosphatidate cytidylyltransferase (CDP-DAGS). The enzyme PGPS converts this CDP-DAG to PGP, which undergoes dephosphorylation to form phosphotidyl glycerol; a molecule CDP-DAG and PtdG is important for the production of membrane lipid CL *a.k.a* diphosphatidylglycerol, indispensible for mitochondrial membrane function and integrity. Within mitochondria, CL is hydrolysed to PA, by action of phospholipases (-A2, -D). Cho, the second product of GPC acylation reaction, is phosphorylated by CHKA to form PCho, which is further acted by PCho CT to form CDP-Cho. The GPC and PCho are major cytoplasmic choline reserves. CDPCho by action of PCT-DAG choline phosphotransferase is converted to which by action of PLC is changed to DAG. DAG facilitates activation of PKC that supplements cell survival in cancer. DAG most importantly yields PtdEtn which constitutes 15–20% of total MPL, and PtdCho through the Kennedy pathway, which together contribute to around 50% cumulative phospholipids content in mammalian cell membrane. PtdEtn is mainly formed from CDP ethanolamine and phosphatidylserine decarboxylase pathways. PtdIns [3,4,5] triphosphate stimulates protein kinase AKT which participates in cell growth and survival, and is also a resident of phospholipid cell membrane. PtdSer is formed from Base Exchange reactions with PtdEtn and PtdCho. Alternatively, DAG can also form PA by DAGK, and DAG is used for TAG synthesis by DGAT. TAG and lipid droplets are storage form of lipids in cytoplasm. The dietary, exogenous FA arising from blood stream are channeled through cell membrane FA binding protein "CD36" into the cytoplasmic FFA pool; FA also arises *de novo* from MAG. The FA make up the glycerol backbones at *sn*-1 and *sn*-2 position for the glycero-phospholipid structure and phosphate group forms the head structure in head-tail architecture of glycero-phospholipids at the *sn*-3 position. G3P in phospholipid metabolism can also be formed from the glycolytic pathway from the product dihydroxyacetone phosphate, and the cellular acetyl CoA central to phospholipid synthesis is chiefly formed from citrate arising from tricarboxylic acid cycle (TCA) *a.k.a* Kreb cycle by action of ACLY; hence phospholipid— choline metabolism is also intimately connected to the energy metabolism, glycolysis and TCA cycle. GPC, glycerophosphocholine GPC; Cho, choline; EDI3, endometrial carcinoma differential 3; G3P, glycerol-3-phosphate; GPAT, glycerol-3-phosphate acyltransferase; LPA, lysophosphatidic acid; PA, phosphatidic acid; PLA, phospholipase A; CDP-DAG, cytidine diphosphate diacylglycerol; PGPS, phosphatidyl glycerol synthase; PGP, phosphatidyl glycerol phosphate; CL, cardiolipins; CT, cytidyl transferase; PtdG, phosphatidyl glycerol; PLC, phospholipase C; CDP-Cho, CDPCho-cytidine 5'-diphosphocholine; DAG, diacylglycerol; PKC, protein kinase C; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; MPL, membrane phospholipids; PtdSer, phosphatidylserine; PtdIn, phosphatidylinositol; DAGK, DAG kinase; TAG, triacylglycerol; DGAT, diacylglycerol acyltransferase; FA, fatty acids; FFA, free fatty acid; MAG, monoacyl glycerol; TCA, tricarboxylic acid cycle; ACLY, ATP citrate lyase.

to decreased cellular attachment and spreading accompanied by delayed formation of cellular protrusions (16). The tumors overexpressing EDI3 showed higher risk of metastasis and decreased survival, linking this protein to metastasis and survival (16).

But putative downstream mediators of EDI3 were recently exposed by the RM group as being mediated via GPAT (17) (*Figure 2*). Among 13 cancer subtypes through bioinformatics approach, high ectopic *GPAT2* expression has been identified by Baro group in a range of human cancers: melanoma, lung, prostate and breast cancer and relatively low expression was identified in renal, colorectal, hepatocellular, basal cell and hematological cancers (10). Before this, GPAT2 has been identified in multiple myelomas. Moreover the absence of GPAT2 produces induced apoptosis (9). *GPAT2* knockdown and overexpression studies showed significant effect on cell proliferation, anoikis, migration and tumorigenicity, and staurosporine-induced apoptosis in several cell types (10). The increased expression of *GPAT2* with a methyltransferase inhibitor suggested epigenetic regulation as its underlying mechanism. Overall, *GPAT2* is strongly adherent to malignancy than glycerolipid synthesis, functionwise slightly different from its isoforms (10). The mechanism of activation of *GPAT2* in spermatogenesis and its ectopic overexpression in cancer is through demethylation, i.e., epigenetic activation. Glycerophosphodiesterase EDI3 has been suggested to promote cell migration, adhesion and spreading, by the recent study of RM group (17). RM group targeted the GPAT1 along with choline kinase-α (CHKA), the enzymes that catabolize the products of EDI3-"G3P and choline" respectively, to determine which potential downstream pathways are precisely responsible for cellular migration (17). Their results clearly indicate that GPAT1 influences cell migration through "LPA signaling". In the recent study, high *GPAT1* expression was also observed in ovarian cancer patients with diminished survival. Also gene silencing experiments in ovarian cancer cell lines decreased cellular migration and growth of tumor xenografts (17). Favoring these observations, manipulating CHKA did not influence cell migration in the same set of cell lines. Based on these potential findings, RM group identified that GPAT1 influences intracellular LPA levels thereby promoting cell migration and tumor growth. Recently, LPA, the product of GPAT1, was also recently identified as essential to mitochondrial fusion, and *GPAT1* mutants (C. elegans animal and Hela cell model) displayed mitochondrial fragmentation (18). Disturbed mitochondrial parameters like mitochondrial fusion rate has been linked to cancer; however the contribution of this mechanism to cellular migration was not exposed with respect to GPAT1 by RM group colleagues.

Under normal conditions, mitochondrial morphology is governed by continuous fusion and fission events. Coleman group identified transgenic mice with GPAT1 deficiency to show reduced susceptibility to hepatocellular carcinoma from exposure to diethylnitrosamine and phenobarbital (7), adding weight to fundamental role of elevated GPAT1 to lipid synthesis in cancer. Recently through transcriptome derived datasets, a range of phospholipid breakdown products (including LPA) and eicosanoids were identified among a panel of mediators in ovarian tumor cells and tumor associated macrophages obtained through ascites sampling. Monitoring the levels of LPA for example may be helpful in early identification of relapse cases and tumor aggressiveness (prognostic markers?). It is of considerable interest that different LPA sps exert different biological effect and their characterization (pertained to FA-sn1 position) may be more helpful.

Choline metabolites, phosphocholine (PCho) and choline are universal to human cancers and have also been used to monitor increased tCho signal via ^{1}H magnetic resonance spectroscopy and choline PET, allowing molecular imaging for early cancer detection (19). Metabolic products have been linked to cancer development and choline metabolism especially has to be considered as a potential cancer target.

Pharmacological inhibition of GPAT in cancer management

We appreciate the elaborate work of RM group who identified potential lipidomic pathways responsible for cancer metastasis. Dissecting such potential pathways/achilles heel in cancer is challenging and critical for drug design against enzymes catalyzing rate limiting steps. Uncontrolled proliferation and cellular migration are classic features in all human cancers, and their arrest is possible through blockage of key phospholipid enzymes like *GPAT1*. This may be possible by the antagonists of GPATeg: *FSG67* (GPAT-1,-2 inhibitor), 2-(nonylsulfonamido) benzoic acid (15 g) (5,20). Based on available evidence design of small molecule inhibitors against GPAT may be effective for cancer therapeutics besides their roles in control of chronic diseases like obesity and type 2 diabetes. The same result would also be achieved by the blockage of type 1 LPA receptor (e.g., Ki16425) in cancer cells, which was already shown to reduce bony metastasis in animal models (12).

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Footnote

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