

Monoacylglycerol Lipase Regulates a Fatty Acid Network that Promotes Cancer Pathogenesis

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DOI 10.1016/j.cell.2009.11.027

SUMMARY

Tumor cells display progressive changes in metabolism that correlate with malignancy, including development of a lipogenic phenotype. How stored fats are liberated and remodeled to support cancer pathogenesis, however, remains unknown. Here, we show that the enzyme monoacylglycerol lipase (MAGL) is highly expressed in aggressive human cancer cells and primary tumors, where it regulates a fatty acid network enriched in oncogenic signaling lipids that promotes migration, invasion, survival, and in vivo tumor growth. Overexpression of MAGL in nonaggressive cancer cells recapitulates this fatty acid network and increases their pathogenicity—phenotypes that are reversed by an MAGL inhibitor. Impairments in MAGL-dependent tumor growth are rescued by a high-fat diet, indicating that exogenous sources of fatty acids can contribute to malignancy in cancers lacking MAGL activity. Together, these findings reveal how cancer cells can co-opt a lipolytic enzyme to translate their lipogenic state into an array of protumorigenic signals.

INTRODUCTION

The conversion of cells from a normal to cancerous state is accompanied by reprogramming of metabolic pathways (DeBerardinis et al., 2008b; Jones and Thompson, 2009; Kroemer and Pouyssegur, 2008), including those that regulate glycolysis (Christofk et al., 2008; Gatenby and Gillies, 2004), glutamine-dependent anaplerosis (DeBerardinis et al., 2007, 2008a; Wise et al., 2008), and the production of lipids (DeBerardinis et al., 2008a; Menendez and Lupu, 2007). Despite a growing appreciation that dysregulated metabolism is a defining feature of cancer, it remains unclear, in many instances, how such biochemical changes occur and whether they play crucial roles

in disease progression and malignancy. Answers to these questions are important for identifying metabolic pathways that are vital to the pathogenesis of cancer.

Among dysregulated metabolic pathways, heightened de novo lipid biosynthesis, or the development of a “lipogenic” phenotype (Menendez and Lupu, 2007), has been posited to play a major role in cancer. For instance, elevated levels of fatty acid synthase (FAS), the enzyme responsible for fatty acid biosynthesis from acetate and malonyl CoA, are correlated with poor prognosis in breast cancer patients, and inhibition of FAS results in decreased cell proliferation, loss of cell viability, and decreased tumor growth in vivo (Kuhajda et al., 2000; Menendez and Lupu, 2007; Zhou et al., 2007). FAS may support cancer growth, at least in part, by providing metabolic substrates for energy production (via fatty acid oxidation) (Buzzai et al., 2005, 2007; Liu, 2006). Many other features of lipid biochemistry, however, are also critical for supporting the malignancy of cancer cells, including (1) the generation of building blocks for newly synthesized membranes to accommodate high rates of proliferation (DeBerardinis et al., 2008a, 2008b), (2) the composition and regulation of membrane structures that coordinate signal transduction and motility (e.g., lipid rafts [Gao and Zhang, 2008], invadopodia [Stylli et al., 2008], blebs [Fackler and Grosse, 2008]), and (3) the biosynthesis of an array of protumorigenic lipid-signaling molecules. Prominent examples of lipid messengers that contribute to cancer include (1) phosphatidylinositol-3,4,5-trisphosphate [PI(3,4,5)P₃], which is formed by the action of phosphatidylinositol-3-kinase and activates protein kinase B/Akt to promote cell proliferation and survival (Yuan and Cantley, 2008; Zunder et al., 2008); (2) lysophosphatidic acid (LPA), which signals through a family of G protein-coupled receptors to stimulate cancer aggressiveness (Mills and Mooleenaar, 2003; Ren et al., 2006); and (3) prostaglandins formed by cyclooxygenases, which support migration and tumor-host interactions (Gupta et al., 2007; Marnett, 1992).

Lipogenesis may thus contribute to cancer by multiple mechanisms. Considering, however, that newly synthesized fatty acids are rapidly incorporated into neutral- and phospho-lipid stores (Menendez and Lupu, 2007), each of the aforementioned models necessitates that cancer cells also possess a

complementary “lipolytic” pathway to liberate stored fatty acids for metabolic and signaling purposes (Prentki and Madiraju, 2008; Przybytkowski et al., 2007).

Here, we use functional proteomic methods to discover a lipolytic enzyme, monoacylglycerol lipase (MAGL), that is highly elevated in aggressive cancer cells from multiple tissues of origin. We show that MAGL, through hydrolysis of monoacylglycerols (MAGs), controls free fatty acid (FFA) levels in cancer cells. The resulting MAGL-FFA pathway feeds into a diverse lipid network enriched in protumorigenic signaling molecules and promotes migration, survival, and in vivo tumor growth. Aggressive cancer cells thus pair lipogenesis with high lipolytic activity to generate an array of protumorigenic signals that support their malignant behavior.

RESULTS

Activity-Based Proteomic Analysis of Hydrolytic Enzymes in Human Cancer Cells

To identify enzyme activities that contribute to cancer pathogenesis, we conducted a functional proteomic analysis of a panel of aggressive and nonaggressive human cancer cell lines from multiple tumors of origin, including melanoma (aggressive [C8161, MUM2B], nonaggressive [MUM2C]), ovarian (aggressive [SKOV3], nonaggressive [OVCAR3]), and breast (aggressive [231MFP], nonaggressive [MCF7]) cancer. Aggressive cancer lines were confirmed to display much greater in vitro migration and in vivo tumor-growth rates compared to their nonaggressive counterparts (Figure S1 available online), as previously shown (Jessani et al., 2002, 2004; Seftor et al., 2002; Welch et al., 1991). Proteomes from these cancer lines were screened by activity-based protein profiling (ABPP) using serine hydrolase-directed fluorophosphonate (FP) activity-based probes (Jessani et al., 2002; Patricelli et al., 2001). Serine hydrolases are one of the largest and most diverse enzyme classes in the human proteome (representing ~1%–1.5% of all human proteins) and play important roles in many biochemical processes of potential relevance to cancer, such as proteolysis (McMahon and Kwaan, 2008; Puustinen et al., 2009), signal transduction (Puustinen et al., 2009), and lipid metabolism (Menendez and Lupu, 2007; Zechner et al., 2005). The goal of this study was to identify hydrolytic enzyme activities that were consistently altered in aggressive versus nonaggressive cancer lines, working under the hypothesis that these conserved enzymatic changes would have a high probability of contributing to the pathogenic state of cancer cells.

Serine hydrolase activities were identified from aggressive and nonaggressive cancer cell proteomes by enrichment with a biotinylated FP probe (Liu et al., 1999) and multidimensional liquid chromatography-mass spectrometry analysis (Jessani et al., 2005). Among the more than 50 serine hydrolases detected in this analysis (Tables S1, S2, and S3), two enzymes, KIAA1363 and MAGL, were found to be consistently elevated in aggressive cancer cells relative to their nonaggressive counterparts, as judged by spectral counting (Jessani et al., 2005; Liu et al., 2004). We confirmed elevations in KIAA1363 and MAGL in aggressive cancer cells by gel-based ABPP, where proteomes are treated with a rhodamine-tagged FP probe and resolved by

1D-SDS-PAGE and in-gel fluorescence scanning (Figure 1A). In both cases, two forms of each enzyme were detected (Figure 1A), due to differential glycosylation for KIAA1363 (Jessani et al., 2002), and possibly alternative splicing for MAGL (Karlsson et al., 2001). We have previously shown that KIAA1363 plays a role in regulating ether lipid signaling pathways in aggressive cancer cells (Chiang et al., 2006). On the other hand, very little was known about the function of MAGL in cancer.

The heightened activity of MAGL in aggressive cancer cells was confirmed using the substrate C20:4 MAG (Figure 1B). Since several enzymes have been shown to display MAG hydrolytic activity (Blankman et al., 2007), we confirmed the contribution that MAGL makes to this process in cancer cells using the potent and selective MAGL inhibitor JZL184 (Long et al., 2009a). JZL184 (1 μ M, 4 hr) dramatically reduced the MAG hydrolytic activity of cancer cells (Figure 1B) and selectively blocked the FP-rhodamine signals for both the 33 and 35 kDa forms of MAGL (Figure 1A). In contrast, JZL184 treatment did not alter the hydrolytic activity displayed by cancer cells for several additional classes of lipids, including diacylglycerols, triacylglycerols, lysophospholipids, and phospholipids (Figure S1). These data demonstrate that aggressive cancer cells display highly elevated MAG hydrolytic activity and most, if not all, of this activity originates from the MAGL enzyme.

MAGL Regulates Free Fatty Acid Levels in Aggressive Cancer Cells

MAGL is perhaps best recognized for its role in degrading the endogenous cannabinoid 2-arachidonoylglycerol (2-AG, C20:4 MAG), as well as other MAGs, in brain and peripheral tissues (Dinh et al., 2002; Long et al., 2009a, 2009b; Nomura et al., 2008). Consistent with this established function, blockade of MAGL by JZL184 (1 μ M, 4 hr) produced significant elevations in the levels of several MAGs, including 2-AG, in each of the aggressive cancer cell lines (Figure 1C and Figure S2). Interestingly, however, MAGL inhibition also caused significant reductions in the levels of FFAs in aggressive cancer cells (Figure 1D and Figure S2). This surprising finding contrasts with the function of MAGL in normal tissues, where the enzyme does not, in general, control the levels of FFAs (Long et al., 2009a, 2009b; Nomura et al., 2008).

Curiously, we noted that, with the exception of C20:4 FFA and MAG, the magnitude of reduction of FFAs greatly exceeded the corresponding elevation in MAGs (~5000–6100 pmol and 41–75 pmol, respectively, for C16:0, C18:0, and C18:1 lipids). We hypothesized that this apparent discrepancy in mass balance might be accounted for by the conversion of elevated MAGs to alternative metabolites in JZL184-treated cancer cells. Consistent with this premise, lipidomic analyses revealed significant increases in two major classes of lysophospholipids—lysophosphatidyl choline (LPC) and lysophosphatidyl ethanolamine (LPE)—in JZL184-treated cancer cells (Figure S1 and Table S4). The cumulative magnitude of elevation of these lysophospholipids (4100–6500 pmol) matched closely the reduction in FFAs observed in JZL184-treated cells. Notably, we did not detect C20:4 lysophospholipids in cancer cells, providing a likely explanation for why JZL184 caused similar magnitudes of elevation and reduction in C20:4 MAG and FFA, respectively.

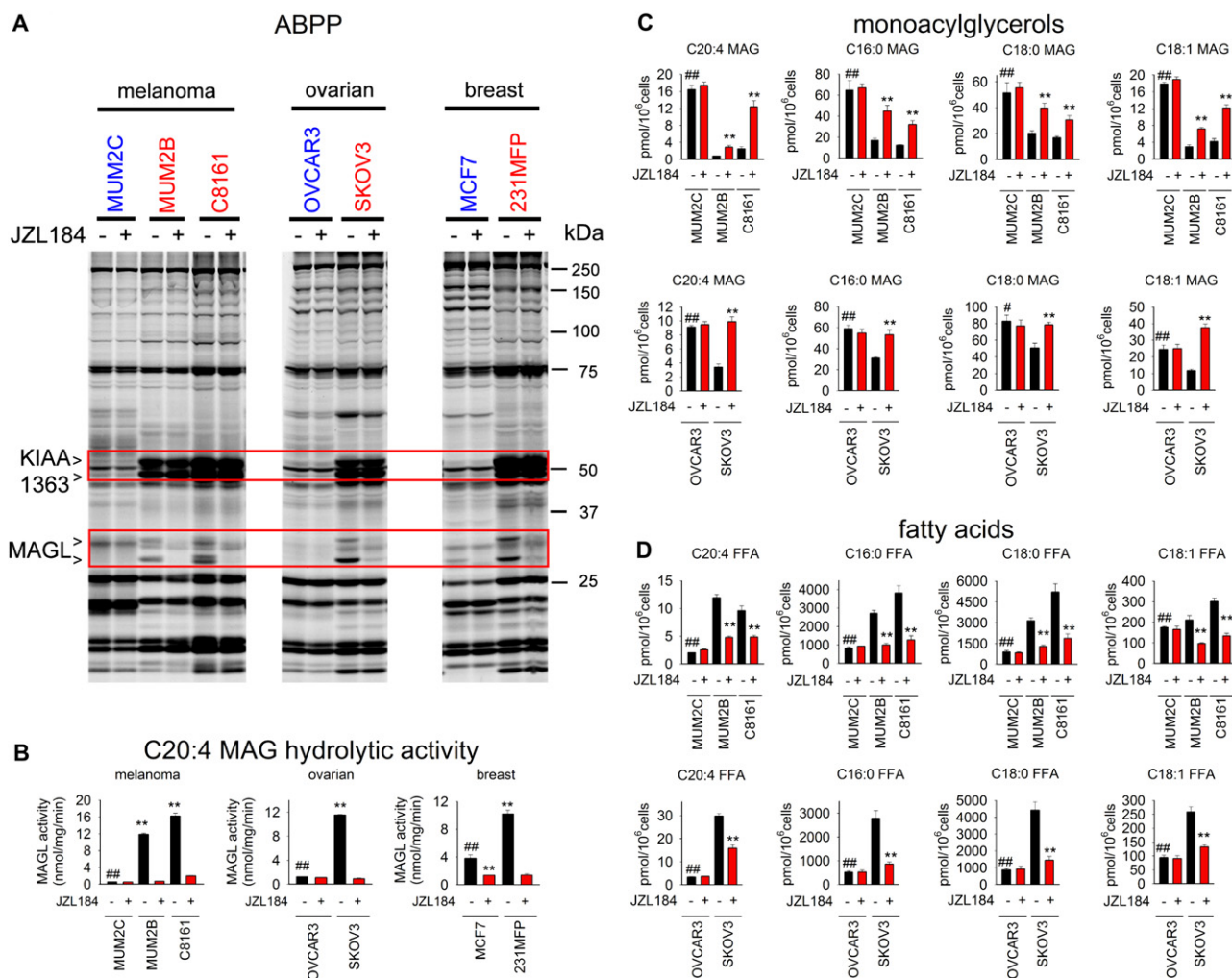


Figure 1. MAGL Is Elevated in Aggressive Cancer Cells, where the Enzyme Regulates Monoacylglycerol and Free Fatty Acid Levels

(A) ABPP of serine hydrolase activities in nonaggressive (blue) and aggressive (red) human cancer cell lines. Serine hydrolase activities were labeled in whole-cell proteomes with the activity-based probe FP-rhodamine and detected by SDS-PAGE and in-gel fluorescence scanning (fluorescent gel shown in grayscale). Highlighted in red boxes are two enzymes, MAGL and KIAA1363 that are consistently elevated in aggressive versus nonaggressive cancer cells. Proteomes were also prepared from cancer cells pretreated with DMSO or the selective MAGL inhibitor JZL184 (1 μ M, 4 hr) to confirm that the 33 and 35 kDa FP-rhodamine-labeled bands represented MAGL. See Table S1, Table S2, and Table S3 for a global analysis of FP-biotin-labeled serine hydrolase activities detected by ABPP in cancer cells.

(B) C20:4 MAG hydrolytic activity of cancer cells in the presence (red bars) or absence (black bars) of JZL184 (1 μ M, 4 hr).

(C and D) Inhibition of MAGL (JZL184 1 μ M, 4 hr) raises MAG (C) and lowers FFA (D) levels in aggressive but not nonaggressive cells. See Table S4 for further metabolite analysis of JZL184-treated cells. Note that aggressive cancer cells possess basally higher levels of FFAs (and lower levels of MAGs) compared to nonaggressive cancer cells, reflecting their respective MAGL activities. * $p < 0.05$, ** $p < 0.01$ for JZL184-treated versus DMSO-treated control groups. # $p < 0.05$, ## $p < 0.01$ for aggressive versus nonaggressive cancer cells. Data are presented as means \pm standard error of the mean (SEM); $n = 4$ –5/group.

Metabolic labeling studies using the non-natural C17:0-MAG confirmed that MAGs are converted to LPC and LPE by aggressive cancer cells, and that this metabolic transformation is significantly enhanced by treatment with JZL184 (Figure S1). Finally, JZL184 treatment did not affect the levels of MAGs and FFAs in nonaggressive cancer lines (Figures 1C and 1D), consistent with the negligible expression of MAGL in these cells (Figures 1A and 1B).

We next stably knocked down MAGL expression by RNA interference technology using two independent shRNA probes

(shMAGL1, shMAGL2), both of which reduced MAGL activity by 70%–80% in aggressive cancer lines (Figures 2A and 2D and Figure S2). Other serine hydrolase activities were unaffected by shMAGL probes (Figures 2A and 2D and Figure S2), confirming the specificity of these reagents. Both shMAGL probes caused significant elevations in MAGs and corresponding reductions in FFAs in aggressive melanoma (Figures 2B and 2C), ovarian (Figures 2E and 2F), and breast cancer cells (Figure S2).

Together, these data demonstrate that both acute (pharmacological) and stable (shRNA) blockade of MAGL cause elevations

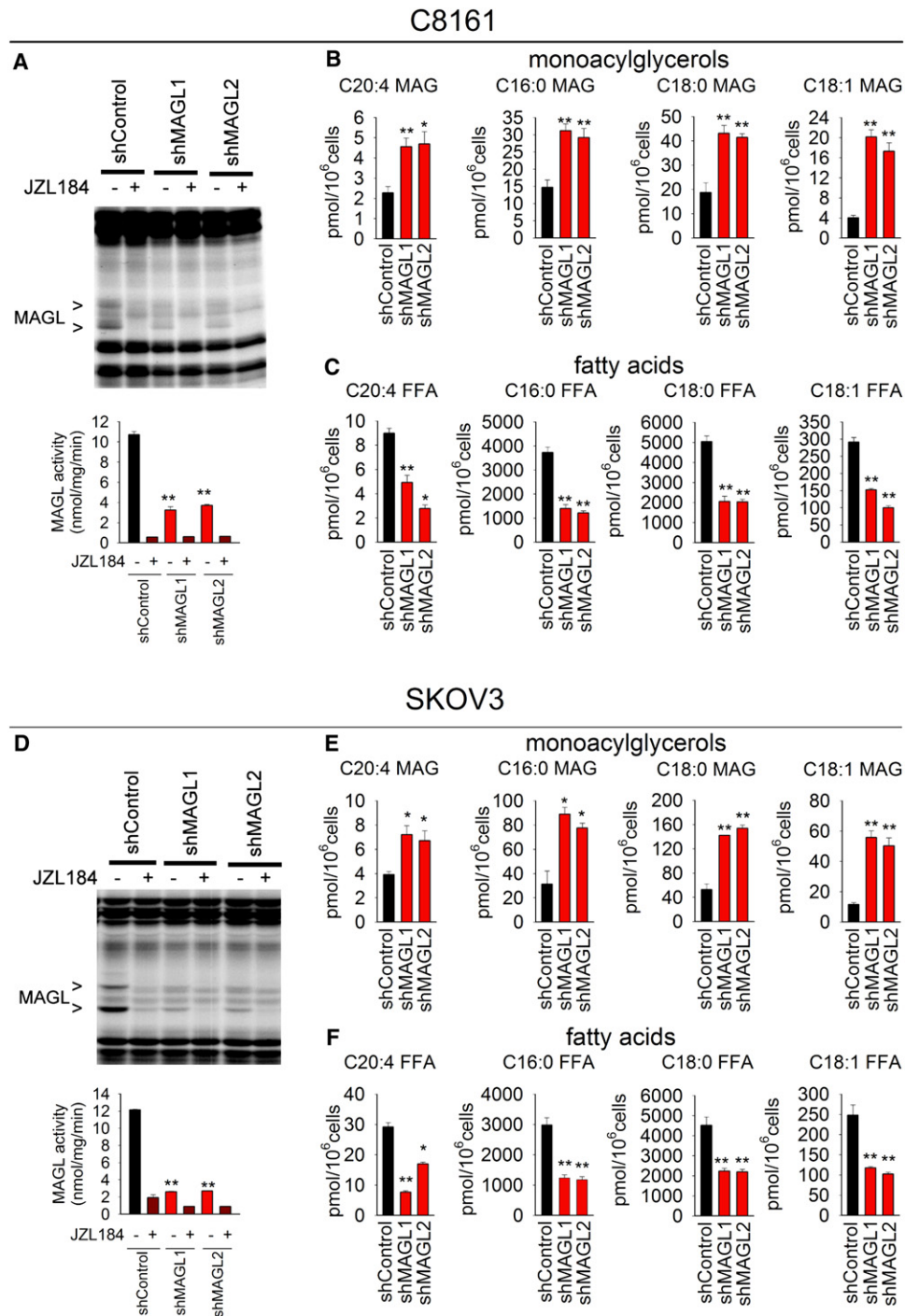


Figure 2. Stable shRNA-Mediated Knockdown of MAGL Lowers FFA Levels in Aggressive Cancer Cells

(A and D) MAGL was stably knocked down using two independent short-hairpin RNA (shRNA) oligonucleotides (shMAGL1, shMAGL2), resulting in >70% reductions in MAGL activity in C8161 and SKOV3 cells compared to shControl cells expressing an shRNA that targets a distinct serine hydrolase (DPP4V).

(B, C, E, and F) shMAGL cells show elevations in MAG (B and E) and reductions in FFA (C and F) levels. * $p < 0.05$, ** $p < 0.01$ for shMAGL versus shControl groups. # $p < 0.05$, ## $p < 0.01$ for aggressive versus nonaggressive cancer cells. The MAGL activity and MAG and FFA levels of shControl cells did not differ significantly from those of parental cancer lines (shown in Figure 1). Data are presented as means \pm SEM; $n = 4$ –5/group. See also Table S4 and Figure S2.

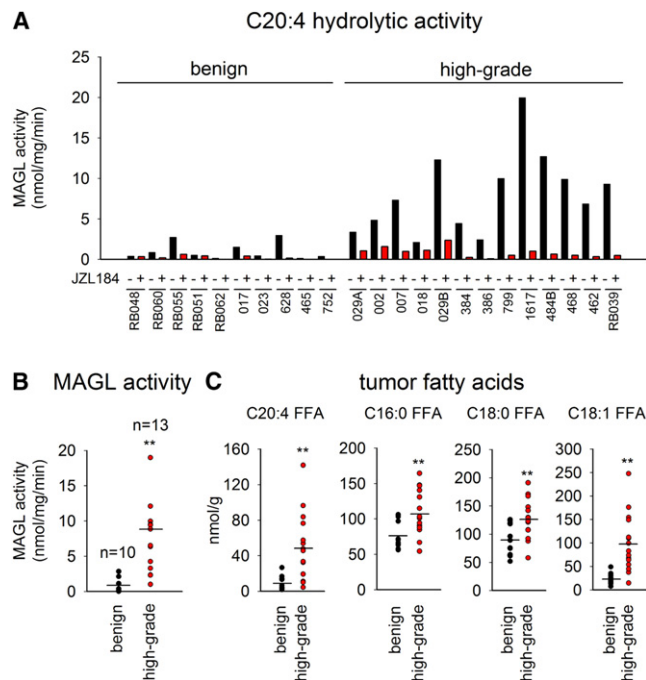


Figure 3. High-Grade Primary Human Ovarian Tumors Possess Elevated MAGL Activity and FFAs Compared to Benign Tumors

(A) C20:4 MAG hydrolytic activity measurements for individual tumor specimens. Pretreatment with JZL184 (1 μ M, 30 min) confirmed that the majority of the observed hydrolytic activity is due to MAGL.

(B) Summary graph of MAGL activity in benign versus high-grade tumors, where each value is expressed as the JZL184-sensitive portion of total C20:4 MAG hydrolytic activity shown in part (A).

(C) Levels of FFAs in benign versus high-grade tumors. ** $p < 0.01$ for high-grade versus benign tumor groups. Data are presented as means \pm SEM; $n = 10$ –13/group.

in MAGs and reductions in FFAs in aggressive cancer cells. It is furthermore noteworthy that aggressive cancer cells were found to express higher basal levels of FFAs (and conversely lower levels of MAGs) than nonaggressive cancer cells (Figures 1C and 1D), and this altered metabolic profile was largely eradicated by MAGL inhibition. These intriguing findings indicate that MAGL is the principal regulator of FFA levels in aggressive cancer cells. Finally, we confirmed that MAGL activity (Figures 3A and 3B) and FFA levels (Figure 3C) are also elevated in high-grade primary human ovarian tumors compared to benign or low-grade tumors. Thus, heightened expression of the MAGL-FFA pathway is a prominent feature of both aggressive human cancer cell lines and primary tumors.

Disruption of MAGL Expression and Activity Impairs Cancer Pathogenicity

shMAGL cancer lines were next examined for alterations in pathogenicity using a set of in vitro and in vivo assays. shMAGL melanoma (C8161), ovarian (SKOV3), and breast (231MFP) cancer cells exhibited significantly reduced in vitro migration (Figures 4A and 4F and Figure S2), invasion (Figures 4B and 4G and Figure S2), and cell survival under serum-starvation conditions

(Figures 4C and 4H and Figure S2). Acute pharmacological blockade of MAGL by JZL184 also decreased cancer cell migration (Figure S2), but not survival, possibly indicating that maximal impairments in cancer aggressiveness require sustained inhibition of MAGL.

shMAGL C8161 and SKOV3 cancer cells also exhibited markedly reduced tumor growth rates in subcutaneous xenograft transplantation studies performed in immune-deficient mice (Figures 4D and 4I). Similar impairments in tumor growth rates were observed in C8161 and SKOV3 xenograft-transplanted mice administered JZL184 once per day (40 mg/kg, per os [p.o.]) (Figures 4E and 4J), a treatment regime that was confirmed to block MAGL activity in tumors (Figure S3). Notably, MAGL-disrupted tumors possessed lower FFA levels (Figure S3), indicating that MAGL maintains its control over fatty acid metabolism in cancer cells grown in vivo.

Collectively, these in vitro and in vivo studies demonstrate that MAGL activity supports several of the aggressive properties exhibited by malignant cancer cells.

MAGL Overexpression Increases FFAs and the Aggressiveness of Cancer Cells

We next asked whether expressing MAGL in nonaggressive cancer cells might alter their lipid metabolic profiles and pathogenicity. Stable MAGL-overexpressing (MAGL-OE) and control (expressing an empty vector or a catalytically inactive version of MAGL, where the serine nucleophile was mutated to alanine [S122A]) variants of MUM2C and OVCAR3 cells were generated by retroviral infection and evaluated by ABPP and C20:4 MAG substrate assays for their respective MAGL activities. Both assays confirmed that MAGL-OE cells possess greater than 10-fold elevations in MAGL activity compared to control cells (Figure 5A and Figure S4). MAGL-OE cells also showed significant reductions in MAGs (Figure 5B and Figure S4) and elevated FFAs (Figure 5C and Figure S4). This altered metabolic profile was accompanied by increased migration (Figure 5D and Figure S4), invasion (Figure 5E and Figure S4), and survival (Figure S4) in MAGL-OE cells. None of these effects were observed in cancer cells expressing the S122A MAGL mutant, indicating that they require MAGL activity. Also consistent with this premise, both the metabolic and pathogenic effects observed in MAGL-OE cells were reversed by a single treatment with JZL184 (1 μ M, 4 hr) (Figure 5 and Figure S4). Finally, MAGL-OE MUM2C cells also showed enhanced tumor growth in vivo compared to control cells (Figure 5F). Notably, the increased tumor growth rate of MAGL-OE MUM2C cells nearly matched that of aggressive C8161 cells (Figure S4), indicating that MAGL is sufficient to induce a highly tumorigenic phenotype in melanoma cells. Collectively, these data indicate that ectopic expression of MAGL in nonaggressive cancer cells elevates their FFA levels and promotes pathogenicity both in vitro and in vivo.

Metabolic Rescue of Impaired Pathogenicity in MAGL-Disrupted Cancer Cells

MAGL could support the aggressiveness of cancer cells by reducing the levels of its MAG substrates, elevating the levels of its FFA products, or both. Among MAGs, the principal signaling molecule is the endocannabinoid 2-AG, which activates

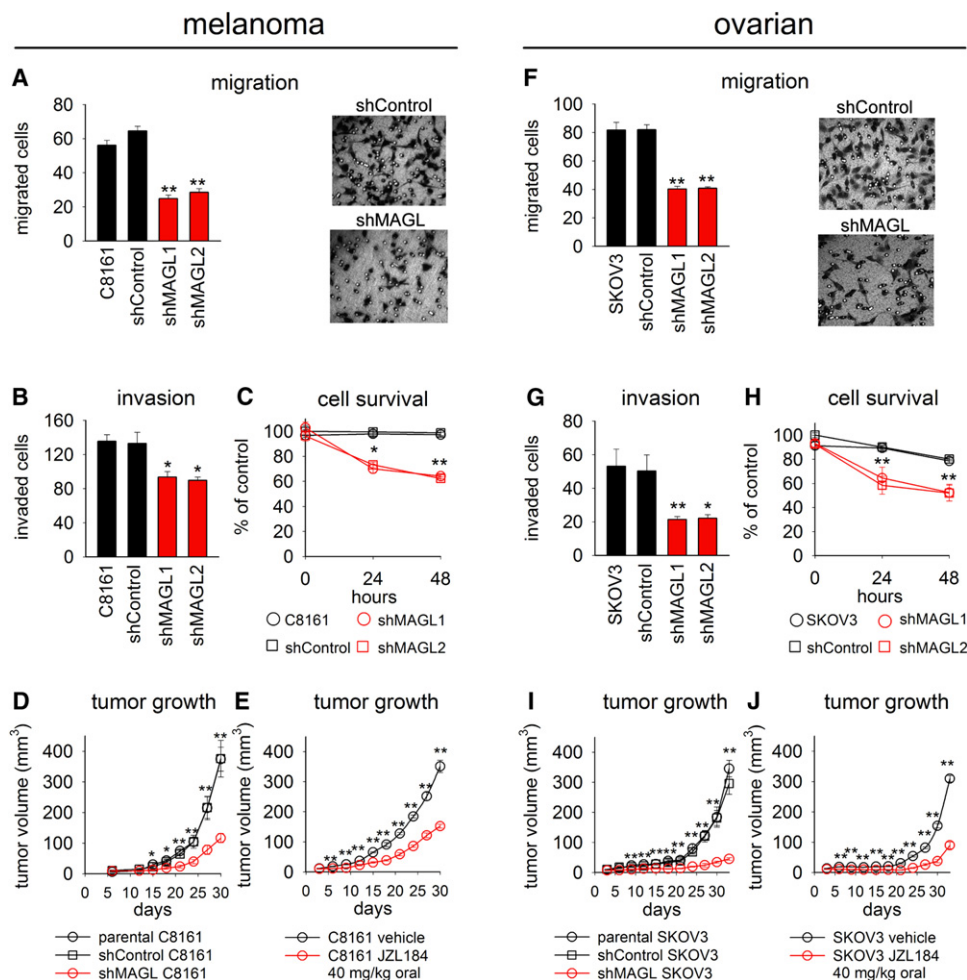


Figure 4. shRNA-Mediated Knockdown and Pharmacological Inhibition of MAGL Impair Cancer Aggressiveness

(A–C and F–H) shMAGL cells show decreased migration (A and F), invasion (B and G), and cell survival (C and H) compared to shControl and uninfected parental cells. Migration and invasion assays were performed by transferring cancer cells to serum-free media for 4 hr prior to seeding 50,000 cells into inserts with 8 μ m pore size containing membranes coated with collagen (10 μ g/ml) or BioCoat Matrigel, respectively. C8161 and SKOV3 migration times were 5 hr and 20 hr, respectively. Migrated or invaded cells refer to average numbers \pm SEM per four fields counted at 400 \times magnification. Cell survival assays were performed by seeding 20,000 cells into 96 well plates in serum-free media. Survival was assessed using the WST-1 proliferation assay. Representative migration plates (at 400 \times magnification) are shown for shControl versus shMAGL cells (A and F).

(D and I) shMAGL cells show impaired tumor growth in SCID mice compared to shControl and uninfected parental cells. 2×10^6 C8161 or SKOV3 cells/100 μ l were injected subcutaneously into the flank and tumor growth was measured using calipers.

(E and J) JZL184 treatment (40 mg/kg daily oral administration in 4 μ l/g polyethylene glycol 300 vehicle) significantly decreases tumor xenograft growth rates in SCID mice compared to vehicle treatment. * $p < 0.05$, ** $p < 0.01$ for shMAGL versus shControl or JZL184 versus vehicle treatment groups. shControl and parental cancer cells did not differ significantly in their migration, cell survival, invasion, or in vivo tumor growth. Data are presented as means \pm SEM; for (A)–(C) and (F)–(H), $n = 5$ –8/group; for (D), (E), (I), and (J), $n = 6$ –11/group. See also Figure S3.

the CB1 and CB2 receptors (Ahn et al., 2008; Mackie and Stella, 2006). The endocannabinoid system has been implicated previously in cancer progression and, depending on the specific study, shown to promote (Sarnataro et al., 2006; Zhao et al., 2005) or suppress (Endsley et al., 2007; Wang et al., 2008) cancer pathogenesis. We therefore tested whether enhanced endocannabinoid signaling (resulting from elevated levels of 2-AG) might mediate the antimigratory effects observed in MAGL-disrupted cancer cells. However, neither a CB1 nor CB2 antagonist rescued the migratory defects of shMAGL cancer cells (Figure S5). CB1 and CB2 antagonists also did not affect the

levels of MAGs or FFAs in cancer cells (Figure S5). These findings, combined with the low expression levels of CB1 and CB2 receptors in aggressive cancer cells (Figure S5), argue that MAGL's effects on cancer aggressiveness were not mediated by endocannabinoid signaling.

Turning our attention to the products of MAGL-catalyzed reactions, we reasoned that, if the protumorigenic effects of MAGL were mediated by FFAs (or their secondary metabolites), then the impaired pathogenicity of shMAGL cancer cells might be rescued by treatment with exogenous sources of fatty acids. In support of this premise, addition of palmitic or stearic acid

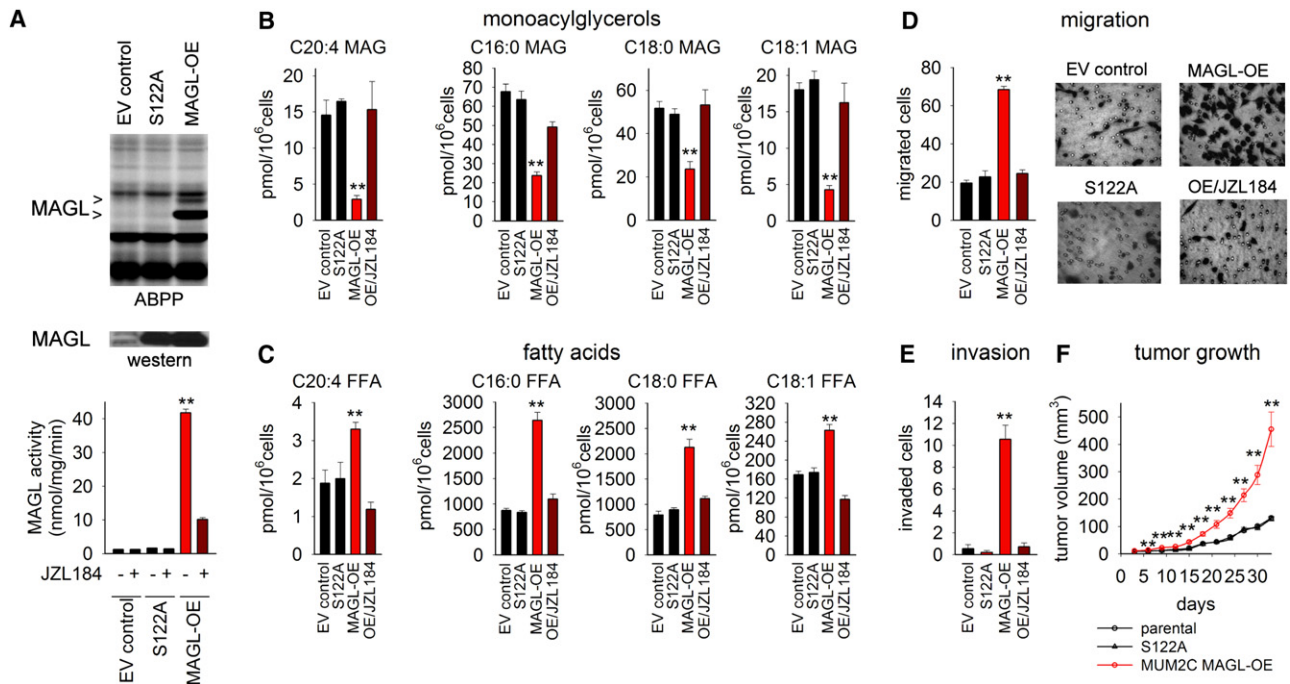


Figure 5. Ectopic Expression of MAGL Elevates FFA Levels and Enhances the In Vitro and In Vivo Pathogenicity of MUM2C Melanoma Cells

(A) MAGL overexpression (MAGL-OE, red bars) in MUM2C cells confirmed by ABPP (top panel), western blot (middle panel), and C20:4 MAG hydrolytic activity (bottom panel). Control and S122A cells (black bars) correspond to cancer cells infected with empty vector (EV) or a catalytically inactive MAGL mutant (S122A), respectively. Western analysis confirmed the overexpression of the S122A-MAGL mutant, which did not show any activity as judged by ABPP and C20:4 MAG hydrolysis assays.

(B and C) MAGL-OE cells contain lower MAG (B) and higher FFA (C) levels compared to EV control and S122A cells. These metabolic effects were reversed by in situ treatment with JZL184 (1 μ M, 4 hr, maroon bars).

(D and E) MAGL-OE MUM2C cells show increased migration (D) and invasion (E) compared to EV and S122A control cells. This enhanced migration and invasion was reversed by JZL184 (1 μ M, 4 hr). Representative migration panels are shown (D).

(F) MAGL-OE MUM2C cells show a significantly enhanced tumor growth rate compared to EV or S122A control cells in SCID mice (orthotopically implanted with 2×10^6 cells). * $p < 0.05$, ** $p < 0.01$ for MAGL-OE versus control groups. Data are presented as means \pm SEM; for (A)–(E), $n = 4$ /group; for (F), $n = 6$ /group. See also Table S4 and Figure S4.

(C16:0 and C18:0 FFAs, respectively; 20 μ M, 4 hr), two principal FFAs regulated by MAGL in aggressive cancer cells, to shMAGL or JZL184-treated MAGL-OE cancer cells fully restored their migratory activity (Figures 6A and 6B and Figure S2). C16:0 and C18:0 FFAs were also found to stimulate the migratory activity of the nonaggressive cancer cells MUM2C and OVCAR3 (Figure 6B). We then determined whether increased FFA delivery could rectify the tumor growth defect observed for shMAGL cells in vivo. Immune-deficient mice were fed either a normal chow or high-fat diet throughout the duration of a xenograft tumor growth experiment. Notably, the impaired tumor growth rate of shMAGL-C8161 cells was completely rescued in mice fed a high-fat diet. In contrast, shControl-C8161 cells showed equivalent tumor growth rates on a normal versus high-fat diet. The recovery in tumor growth for shMAGL-C8161 cells in the high-fat diet group correlated with significantly increased levels of FFAs in excised tumors (Figure 6D).

Collectively, these results indicate that MAGL supports the pathogenic properties of cancer cells by maintaining tonically elevated levels of FFAs. We next asked whether this metabolic profile might impact the larger lipid networks of aggressive cancer cells.

MAGL Regulates a Fatty Acid Network Enriched in Protumorigenic Signals

We first considered whether the MAGL-FFA pathway might serve as a means to regenerate NAD⁺ (via continual fatty acyl glyceride/FFA recycling) to fuel glycolysis, which has been hypothesized to explain a need for elevated neutral lipid hydrolyase activity in cancers (Przybytkowski et al., 2007). Arguing against this model, though, the NAD⁺/NADH ratios and pyruvate and lactate levels were unaltered in shMAGL and MAGL-OE cells relative to control cells (Figure S5). Another possible reason for increased lipolysis could be to generate FFA substrates for β -oxidation, which may serve as an important energy source for cancer cells (Bazzai et al., 2005). However, inhibitors of carnitine palmitoyltransferase 1 (CPT1), which catalyzes the rate-limiting step in β -oxidation (McGarry and Brown, 1997), did not affect the migratory activity of cancer cells (shControl, shMAGL, or MAGL-OE) (Figure S5). CPT1 blockade also failed to alter FFA levels in cancer cells, with the exception of C20:4 FFA, which was elevated only in shControl cells (Figure S5). Additional studies revealed reduced expression of CPT1 in aggressive cancer cells (data not shown), as has been reported previously (Deberardinis et al., 2006), providing further evidence against

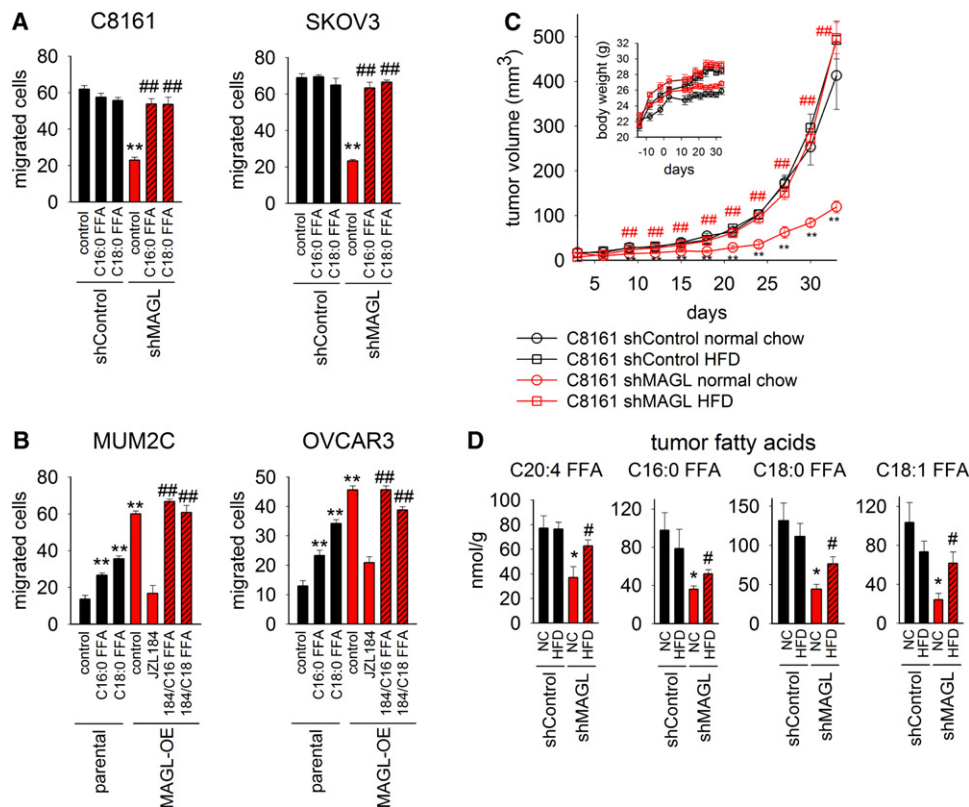


Figure 6. Recovery of the Pathogenic Properties of shMAGL Cancer Cells by Treatment with Exogenous Fatty Acids

(A) The reduced migration of shMAGL cells is reversed by treatment with palmitic or stearic acid (20 μ M, 4 hr, hatched red bars).

(B) Addition of palmitic or stearic acid (20 μ M, 4 hr) increases the migration of MUM2C and OVCAR3 cells and rescues the reduced migration in JZL184-treated MAGL-OE cells.

(C) The reduced tumor growth of shMAGL-C8161 cells is reversed by treatment with a high-fat diet (HFD) (60 kcal% fat). Mice were placed on normal chow (NC) or HFD 2 weeks prior to flank injection of cancer cells and maintained on these diets throughout the tumor growth time course. Inset, body weights for animals throughout time course.

(D) Explanted shMAGL tumors from the HFD group (hatched red bars) contain elevated FFA levels compared to shMAGL tumors from the NC group (red bars). * $p < 0.05$, ** $p < 0.01$ shMAGL versus shControl groups. ### $p < 0.01$ for palmitic or stearic acid- or HFD-treated shMAGL groups versus shMAGL control groups (DMSO- and NC-treated groups, respectively). Data are presented as means \pm SEM; For (A), $n = 4$ –5/group; for (B) and (C), $n = 7$ –8/group. See also Figure S5.

a role for β -oxidation as a downstream mediator of the pathogenic effects of the MAGL-fatty acid pathway.

Considering that FFAs are fundamental building blocks for the production and remodeling of membrane structures and signaling molecules, perturbations in MAGL might be expected to affect several lipid-dependent biochemical networks important for malignancy. To test this hypothesis, we performed lipidomic analyses of cancer cell models with altered MAGL activity, including comparisons of (1) MAGL-OE versus control cancer cells (OVCAR3, MUM2C) and (2) shMAGL versus shControl cancer cells (SKOV3, C8161). Organic extracts of cancer cells were analyzed using an untargeted liquid chromatography-mass spectrometry (LC-MS) platform that profiles several major lipid families (Chiang et al., 2006; Saghatelian et al., 2004), and metabolites with significantly altered levels between the comparison groups were identified using the XCMS software (Smith et al., 2006). Complementing these global profiles, we also conducted targeted measurements of specific bioactive lipids (e.g., prostaglandins) that are too low in abun-

dance for detection by standard lipidomic methods. The resulting data sets were then mined to identify a common signature of lipid metabolites regulated by MAGL, which we defined as metabolites that were significantly increased or reduced in MAGL-OE cells and showed the opposite change in shMAGL cells relative to their respective control groups (Figures 7A and 7B and Table S4).

Most of the lipids in the MAGL-fatty acid network, including several lysophospholipids (LPC, LPA, LPE), ether lipids (MAGE, alkyl LPE), phosphatidic acid (PA), and prostaglandin E2 (PGE₂), displayed similar profiles to FFAs, being consistently elevated and reduced in MAGL-OE and shMAGL cells, respectively. Only MAGs were found to show the opposite profile (elevated and reduced in shMAGL and MAGL-OE cells, respectively). Interestingly, virtually this entire lipidomic signature was also observed in aggressive cancer cells when compared to their nonaggressive counterparts (e.g., C8161 versus MUM2C and SKOV3 versus OVCAR3, respectively; Table S4). These findings demonstrate that MAGL regulates a lipid network in aggressive

cancer cells that consists of not only FFAs and MAGs but also a host of secondary lipid metabolites. We were further intrigued to find that acute inhibition of MAGL by JZL184 produced a distinct secondary lipidomic signature. As was observed in shMAGL cells, JZL184 treatment caused reductions in LPA and PGE₂ in each of the cancer lines expressing high levels of MAGL (Figures 7C and 7D and Table S4). In contrast, and as noted earlier, increases (rather than decreases) in LPCs and LPEs were observed in JZL184-treated cells (Figure S1 and Table S4). These data indicate that acute and chronic blockade of MAGL generate distinct metabolomic effects in cancer cells, likely reflecting the differential outcomes of short- versus long-term depletion of FFAs. Finally, several additional classes of lipids, including phosphatidylcholines, phosphatidylethanolamines, ceramides, sphingosine-1-phosphate, cholesterol, diacylglycerols, and triacylglycerols, were not affected by reductions or elevations in MAGL activity (Table S4), thus underscoring the restricted composition of the fatty acid network regulated by this enzyme.

Within the MAGL-fatty acid network are several protumorigenic lipid messengers, including LPA and PGE₂, that have been shown to promote the aggressiveness of cancer cells (Gupta et al., 2007; Mills and Moolenaar, 2003). Metabolic labeling studies confirmed that aggressive cancer cells can convert both MAGs and FFAs (Figure S1) to LPA and PGE₂, and for MAGs, this conversion was blocked by JZL184 (Figure S1). Interestingly, treatment with either LPA or PGE₂ (100 nM, 4 hr) rescued the impaired migration of shMAGL cancer cells at concentrations that did not affect the migration of shControl cells (Figure 7E). Conversely, the enhanced migratory activity of MAGL-OE cancer cells was completely blocked by the G_i/G_o inhibitor pertussis toxin (Figure 7F). Finally, the degree of stimulated migration observed in MAGL-OE cells equaled the maximal effect produced by exogenous addition of LPA (Figure 7G). Taken together, these data suggest that MAGL contributes to cancer pathogenicity, at least in part, by elevating the production of bioactive lipids that act on G protein-coupled receptors to promote high migratory activity. To assess whether the MAGL-fatty acid network interacts with other protumorigenic signaling systems, we treated shMAGL and shControl cells with an inhibitor of the epidermal growth factor receptor (EGFR). shMAGL cells show significantly heightened sensitivity to the antimigratory (Figure 7H and Figure S6) and antisurvival (Figure S6) effects of EGFR blockade. These data are consistent with previous findings indicating substantial crosstalk between LPA and EGFR signaling pathways in cancer cells (Bektas et al., 2005; Gschwind et al., 2002).

DISCUSSION

Heightened lipogenesis is an established early hallmark of dysregulated metabolism and pathogenicity in cancer (Menendez and Lupu, 2007). Cancer lipogenesis appears to be driven principally by FAS, which is elevated in most transformed cells and important for survival and proliferation (De Schrijver et al., 2003; Kuhajda et al., 2000; Vazquez-Martin et al., 2008). It is not yet clear how FAS supports cancer growth, but most of the proposed mechanisms invoke protumorigenic functions for the

enzyme's fatty acid products and their lipid derivatives (Menendez and Lupu, 2007). This creates a conundrum because the fatty acid molecules produced by FAS are thought to be rapidly incorporated into neutral and phospho-lipids, pointing to the need for complementary lipolytic pathways in cancer cells to release stored fatty acids for metabolic and signaling purposes (Prentki and Madiraju, 2008; Przybytkowski et al., 2007). Consistent with this hypothesis, we found that acute treatment with the FAS inhibitor C75 (40 μ M, 4 hr) did not reduce FFA levels in cancer cells (data not shown). Furthermore, aggressive and nonaggressive cancer cells exhibited similar levels of FAS (data not shown), indicating that lipogenesis in the absence of paired lipolysis may be insufficient to confer high levels of malignancy.

Here we show that aggressive cancer cells do indeed acquire the ability to liberate FFAs from neutral lipid stores as a consequence of heightened expression of MAGL. MAGL and its FFA products were found to be elevated in aggressive human cancer cell lines from multiple tissues of origin, as well as in high-grade primary human ovarian tumors. These data suggest that the MAGL-FFA pathway may be a conserved feature of advanced forms of many types of cancer. Further evidence in support of this premise originates from gene expression profiling studies, which have identified increased levels of MAGL in primary human ductal breast tumors compared to less malignant medullary breast tumors (Gjerstorff et al., 2006). The key role that MAGL plays in regulating FFA levels in aggressive cancer cells contrasts with the function of this enzyme in normal tissues, where it mainly controls the levels of MAGs, but not FFAs (Long et al., 2009b). These data thus provide a striking example of the co-opting of an enzyme by cancer cells to serve a distinct metabolic purpose that supports their pathogenic behavior.

We determined that MAGL is both necessary and sufficient to elevate FFAs and confer high migratory and tumorigenic activity in cancer cells. Blockade of MAGL impaired not only *in vitro* migration but also *in vivo* tumor growth, and both phenotypes were rescued by exogenous sources of FFAs. These data, in combination with the lack of effect of cannabinoid receptor antagonists on migration, indicate that the mechanism of MAGL-stimulated cancer aggressiveness involves the action of FFA-derived products, rather than reductions in MAGL substrates, such as the endocannabinoid 2-AG. Additional studies argued against a major role for β -oxidation or glycolysis in mediating MAGL-dependent aggressiveness. Instead, we found that MAGL regulates a host of secondary lipid metabolites that include key signaling molecules, such as LPA and PGE₂, known to support cancer malignancy. Our finding that impairments in the MAGL-FFA pathway can be rescued by exogenous fatty acids, including a high-fat diet *in vivo*, has provocative implications for the crosstalk between obesity and tumorigenesis. It has been postulated that excessive fat accumulation may exacerbate the development and progression of cancer (Calle and Kaaks, 2004; Calle et al., 2003), and, conversely, reductions in caloric intake have been shown to impede tumor growth (Kalaany and Sabatini, 2009). Our data suggest one mechanism whereby a high-fat diet might promote malignancy, namely, by stimulating the growth and migratory activity of cancer cells that do not themselves exhibit high rates of lipolysis.

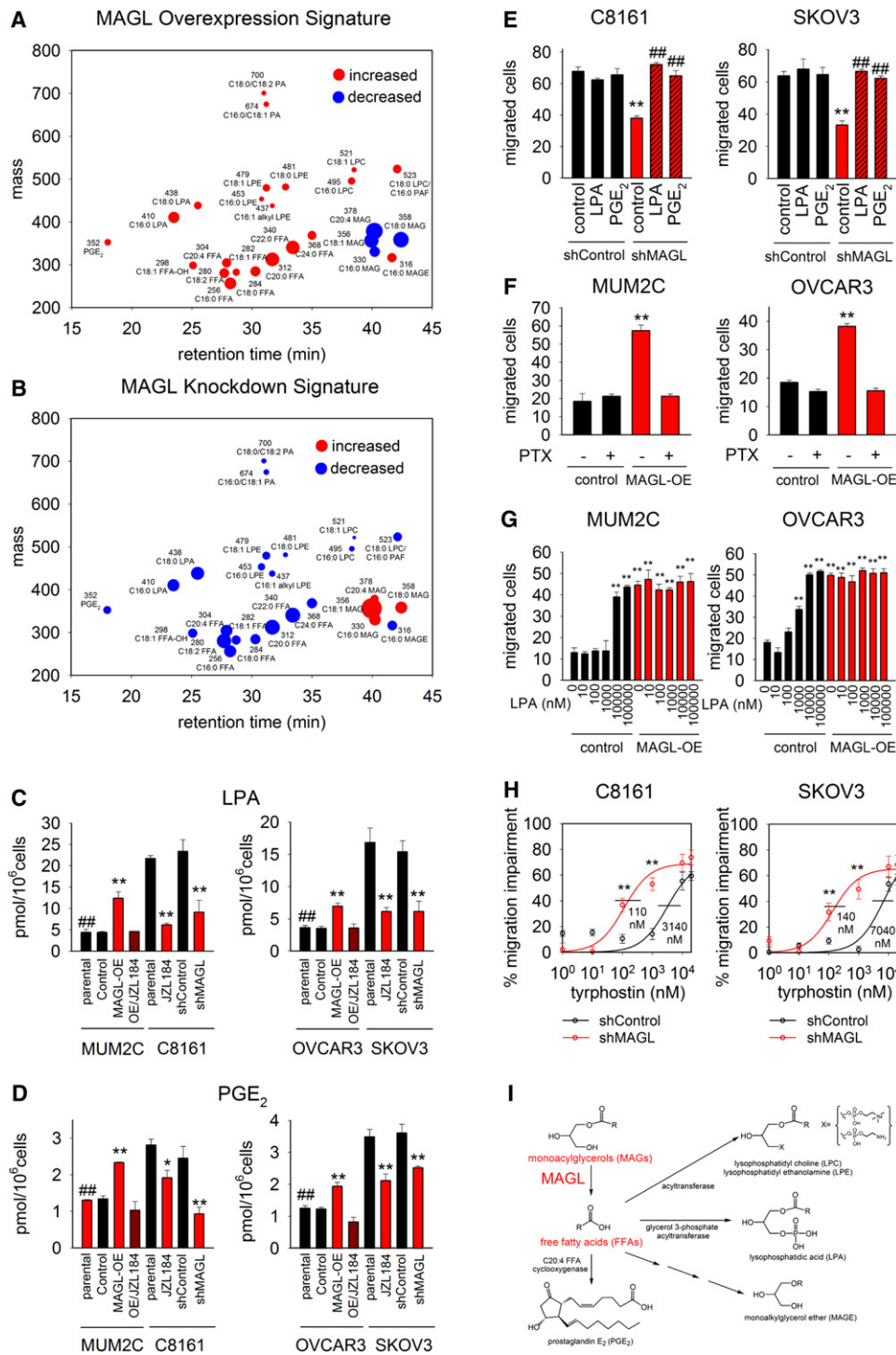


Figure 7. MAGL Regulates a Lipid Network Enriched in Protumorigenic Signaling Molecules

(A and B) Lipidomic analyses of cancer cell models with altered MAGL activity comparing MAGL-OE versus EV control (A) and shMAGL versus shControl (B) cells. The metabolites shown are those that were increased (red) or decreased (blue) in MAGL-OE (both MUM2C and OVCAR3) versus EV control cells (A) and showed the opposite profile in shMAGL (both C8161 and SKOV3) versus shControl cells (B). Parent masses of metabolites are provided, and the size of circles indicates the relative magnitude of change. Quantitation of lipid levels are provided in [Table S4](#).

(C and D) Quantitation of C16:0 LPA and PGE₂ levels in cancer cell models.

(E) Treatment of shMAGL cancer cells with C16:0 LPA (100 nM, 4 hr) or PGE₂ (100 nM, 4 hr) rescues their defective migration compared to shControl cells.

(F) Pertussis toxin (PTX) (100 ng/ml, 4 hr) reverses the increased migration of MAGL-OE cancer cells.

Taken together, our results indicate that MAGL serves as key metabolic hub in aggressive cancer cells, where the enzyme regulates a fatty acid network that feeds into a number of protumorigenic signaling pathways. Additional studies will be required to determine precisely how FFAs are converted to protumorigenic lipid transmitters, although some obvious candidate pathways can be schematized (Figure 7I). It will be interesting to determine whether any of the enzymatic components of these pathways are also dysregulated in pathogenic cancers. One might also anticipate that cancer cells could exhibit heightened levels of additional hydrolytic activities (e.g., di- and tri-acylglycerol hydrolysis) to further capitalize on their lipogenic state, although we should note that the aggressive cancer cells examined herein displayed much lower di- and tri-acylglycerol lipase activity compared to MAGL activity, and these former activities did not differ between aggressive and nonaggressive cancer cells (Figure S1). Finally, considering that endocannabinoids (Wang et al., 2008), β -oxidation (Buzzai et al., 2005; Liu, 2006), and fatty acid-sustained glycolysis (Przybytkowski et al., 2007) have each been described as potential contributing elements to tumorigenesis, it is possible that these pathways may prove relevant for MAGL-dependent aggressiveness in other types of cancer. Independent of which of these mechanisms is operational in specific cancers, our data suggest that they would each function downstream of MAGL, thus designating this enzyme as a potentially exciting pharmacological target for future cancer therapy.

EXPERIMENTAL PROCEDURES

Pharmacological Inhibition of MAGL in Cancer Cells

Pharmacological inhibition studies were conducted as described previously (Chiang et al., 2006). Cells were incubated in serum-free media with JZL184 (1 μ M) or vehicle (DMSO) for 4 hr, after which the cells were harvested and analyzed.

ABPP and Hydrolytic Activity Assays of Cancer Cell Proteomes

Identification and comparative quantitation of serine hydrolase activities from cancer cell proteomes by ABPP-MudPIT or gel-based ABPP were conducted as previously described using FP-biotin (5 μ M) and FP-rhodamine (2 μ M), respectively (Jessani et al., 2002, 2005). C20:4 MAG hydrolytic activity assays were performed as described previously (Blankman et al., 2007).

RNA Interference Studies in Human Cancer Cell Lines

RNA interference studies were conducted as described previously (Chiang et al., 2006). Short-hairpin RNA constructs were subcloned into the pLP-RetroQ acceptor system, and retrovirus was generated by using the Ampho-Pack-293 Cell Line (Clontech).

Overexpression Studies in Human Cancer Cell Lines

Stable MAGL overexpression was achieved by subcloning the MAGL gene into the pMSCVpuro vector (Clontech), generating retrovirus using the Ampho-Pack-293 Cell Lines.

Lipid Measurements in Cancer Cells

Lipid measurements were conducted similarly to protocols described previously (Chiang et al., 2006). Briefly, cell metabolomes were extracted with 2:1:1 chloroform:methanol:Tris buffer. The organic layer was extracted, followed by acidification of the aqueous phase and re-extraction with chloroform. The organic phases were combined, dried under N_2 , and resuspended in chloroform for LC-MS analysis.

Cell Migration, Cell Survival, Invasion, and Tumor Xenograft Studies

Migration, cell survival, invasion, and tumor xenograft studies of cancer cells were performed as described in the Extended Experimental Procedures. Migration assays were performed in Transwell chambers (Corning) coated with 10 μ g/ml collagen. Cell survival assays were performed using the Cell Proliferation Reagent WST-1 (Roche). Invasion assays were conducted using the BD Matrigel Invasion Chambers. Human cancer xenografts were established by transplanting cancer cell lines ectopically into the flank of C.B17 SCID mice.

For more details on the methods, please see the Extended Experimental Procedures available online.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, six figures, and four tables and can be found with this article online at doi:10.1016/j.cell.2009.11.027.

ACKNOWLEDGMENTS

We thank the members of the Cravatt laboratory for helpful discussion and critical reading of the manuscript. In particular, we acknowledge Gabriel M. Simon for assistance with data analysis, Michele K. McKinney, and Jacqueline L. Blankman for technical assistance. This work was supported by the National Institutes of Health (CA132630, CA087660) and the Skaggs Institute for Chemical Biology.

Received: May 5, 2009

Revised: August 18, 2009

Accepted: November 4, 2009

Published: January 7, 2010

REFERENCES

- Ahn, K., McKinney, M.K., and Cravatt, B.F. (2008). Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. *Chem. Rev.* 108, 1687–1707.
- Bektas, M., Payne, S.G., Liu, H., Goparaju, S., Milstien, S., and Spiegel, S. (2005). A novel acylglycerol kinase that produces lysophosphatidic acid modulates cross talk with EGFR in prostate cancer cells. *J. Cell Biol.* 169, 801–811.

(G) Concentration-dependent stimulation of migration by LPA in EV control versus MAGL-OE cells. Note that the maximal stimulation of migration induced by LPA in EV control cells matches the basally enhanced migration observed in MAGL-OE cells, and LPA does not further increase the migration of MAGL-OE cells.

(H) Sensitivity of shControl versus shMAGL cancer cells to the EGFR inhibitor tyrphostin AG-1478, expressed as percentage of migration impairment. The absolute numbers of migrated cells and the effect of tyrphostin on cell survival are shown in Figure S6. IC_{50} values for the antimigratory effects of tyrphostin are provided in the panels.

(I) Scheme showing a possible metabolic network connecting the MAGL-FFA pathway to other protumorigenic lipids.

For (A) and (B), data are presented as mean relative changes between comparison groups; $n = 4$ –5/group. For (C)–(G), * $p < 0.05$, ** $p < 0.01$ for MAGL-OE or shMAGL versus respective control cell groups (C–G), JZL184-treated versus DMSO-treated control cells (C and D). ### $p < 0.01$ for parental nonaggressive (MUM2C, OVCAR3) versus aggressive (C8161, SKOV3) cells (C and D), or LPA/PGE₂-treated shMAGL versus shControl cells (E). Data are presented as means \pm SEM; $n = 3$ –5/group.

- Blankman, J.L., Simon, G.M., and Cravatt, B.F. (2007). A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem. Biol.* 14, 1347–1356.
- Buzzai, M., Bauer, D.E., Jones, R.G., DeBerardinis, R.J., Hatzivassiliou, G., Elstrom, R.L., and Thompson, C.B. (2005). The glucose dependence of Akt-transformed cells can be reversed by pharmacologic activation of fatty acid beta-oxidation. *Oncogene* 24, 4165–4173.
- Buzzai, M., Jones, R.G., Amaravadi, R.K., Lum, J.J., DeBerardinis, R.J., Zhao, F., Viollet, B., and Thompson, C.B. (2007). Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res.* 67, 6745–6752.
- Calle, E.E., and Kaaks, R. (2004). Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nat. Rev. Cancer* 4, 579–591.
- Calle, E.E., Rodriguez, C., Walker-Thurmond, K., and Thun, M.J. (2003). Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N. Engl. J. Med.* 348, 1625–1638.
- Chiang, K.P., Niessen, S., Saghatelian, A., and Cravatt, B.F. (2006). An enzyme that regulates ether lipid signaling pathways in cancer annotated by multidimensional profiling. *Chem. Biol.* 13, 1041–1050.
- Christofk, H.R., Vander Heiden, M.G., Harris, M.H., Ramanathan, A., Gerszten, R.E., Wei, R., Fleming, M.D., Schreiber, S.L., and Cantley, L.C. (2008). The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* 452, 230–233.
- De Schrijver, E., Brusselmans, K., Heyns, W., Verhoeven, G., and Swinnen, J.V. (2003). RNA interference-mediated silencing of the fatty acid synthase gene attenuates growth and induces morphological changes and apoptosis of LNCaP prostate cancer cells. *Cancer Res.* 63, 3799–3804.
- DeBerardinis, R.J., Lum, J.J., and Thompson, C.B. (2006). Phosphatidylinositol 3-kinase-dependent modulation of carnitine palmitoyltransferase 1A expression regulates lipid metabolism during hematopoietic cell growth. *J. Biol. Chem.* 281, 37372–37380.
- DeBerardinis, R.J., Mancuso, A., Daikhin, E., Nissim, I., Yudkoff, M., Wehrli, S., and Thompson, C.B. (2007). Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proc. Natl. Acad. Sci. USA* 104, 19345–19350.
- DeBerardinis, R.J., Lum, J.J., Hatzivassiliou, G., and Thompson, C.B. (2008a). The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab.* 7, 11–20.
- DeBerardinis, R.J., Sayed, N., Ditsworth, D., and Thompson, C.B. (2008b). Brick by brick: metabolism and tumor cell growth. *Curr. Opin. Genet. Dev.* 18, 54–61.
- Dinh, T.P., Freund, T.F., and Piomelli, D. (2002). A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chem. Phys. Lipids* 121, 149–158.
- Endsley, M.P., Aggarwal, N., Isbell, M.A., Wheelock, C.E., Hammock, B.D., Falck, J.R., Campbell, W.B., and Nitthipatikom, K. (2007). Diverse roles of 2-arachidonoylglycerol in invasion of prostate carcinoma cells: Location, hydrolysis and 12-lipoxygenase metabolism. *Int. J. Cancer* 121, 984–991.
- Fackler, O.T., and Grosse, R. (2008). Cell motility through plasma membrane blebbing. *J. Cell Biol.* 181, 879–884.
- Gao, X., and Zhang, J. (2008). Spatiotemporal analysis of differential Akt regulation in plasma membrane microdomains. *Mol. Biol. Cell* 19, 4366–4373.
- Gatenby, R.A., and Gillies, R.J. (2004). Why do cancers have high aerobic glycolysis? *Nat. Rev. Cancer* 4, 891–899.
- Gjerstorff, M.F., Benoit, V.M., Laenkhölm, A.V., Nielsen, O., Johansen, L.E., and Ditzel, H.J. (2006). Identification of genes with altered expression in medullary breast cancer vs. ductal breast cancer and normal breast epithelia. *Int. J. Oncol.* 28, 1327–1335.
- Gschwind, A., Prenzel, N., and Ullrich, A. (2002). Lysophosphatidic acid-induced squamous cell carcinoma cell proliferation and motility involves epidermal growth factor receptor signal transactivation. *Cancer Res.* 62, 6329–6336.
- Gupta, G.P., Nguyen, D.X., Chiang, A.C., Bos, P.D., Kim, J.Y., Nadal, C., Gomis, R.R., Manova-Todorova, K., and Massague, J. (2007). Mediators of vascular remodelling co-opted for sequential steps in lung metastasis. *Nature* 446, 765–770.
- Jessani, N., Liu, Y., Humphrey, M., and Cravatt, B.F. (2002). Enzyme activity profiles of the secreted and membrane proteome that depict cancer cell invasiveness. *Proc. Natl. Acad. Sci. USA* 99, 10335–10340.
- Jessani, N., Humphrey, M., McDonald, W.H., Niessen, S., Masuda, K., Gangadharan, B., Yates, J.R., 3rd, Mueller, B.M., and Cravatt, B.F. (2004). Carcinoma and stromal enzyme activity profiles associated with breast tumor growth in vivo. *Proc. Natl. Acad. Sci. USA* 101, 13756–13761.
- Jessani, N., Niessen, S., Wei, B.Q., Nicolau, M., Humphrey, M., Ji, Y., Han, W., Noh, D.Y., Yates, J.R., 3rd, Jeffrey, S.S., et al. (2005). A streamlined platform for high-content functional proteomics of primary human specimens. *Nat. Methods* 2, 691–697.
- Jones, R.G., and Thompson, C.B. (2009). Tumor suppressors and cell metabolism: a recipe for cancer growth. *Genes Dev.* 23, 537–548.
- Kalaany, N.Y., and Sabatini, D.M. (2009). Tumours with PI3K activation are resistant to dietary restriction. *Nature* 458, 725–731.
- Karlsson, M., Reue, K., Xia, Y.R., Lusis, A.J., Langin, D., Tornqvist, H., and Holm, C. (2001). Exon-intron organization and chromosomal localization of the mouse monoglyceride lipase gene. *Gene* 272, 11–18.
- Kroemer, G., and Pouyssegur, J. (2008). Tumor cell metabolism: cancer's Achilles' heel. *Cancer Cell* 13, 472–482.
- Kuhajda, F.P., Pizer, E.S., Li, J.N., Mani, N.S., Frehywot, G.L., and Townsend, C.A. (2000). Synthesis and antitumor activity of an inhibitor of fatty acid synthase. *Proc. Natl. Acad. Sci. USA* 97, 3450–3454.
- Liu, H., Sadygov, R.G., and Yates, J.R., 3rd. (2004). A model for random sampling and estimation of relative protein abundance in shotgun proteomics. *Anal. Chem.* 76, 4193–4201.
- Liu, Y. (2006). Fatty acid oxidation is a dominant bioenergetic pathway in prostate cancer. *Prostate Cancer Prostatic Dis.* 9, 230–234.
- Liu, Y., Patricelli, M.P., and Cravatt, B.F. (1999). Activity-based protein profiling: the serine hydrolases. *Proc. Natl. Acad. Sci. USA* 96, 14694–14699.
- Long, J.Z., Li, W., Booker, L., Burston, J.J., Kinsey, S.G., Schlosburg, J.E., Pavon, F.J., Serrano, A.M., Selley, D.E., Parsons, L.H., et al. (2009a). Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nat. Chem. Biol.* 5, 37–44.
- Long, J.Z., Nomura, D.K., and Cravatt, B.F. (2009b). Characterization of monoacylglycerol lipase inhibition reveals differences in central and peripheral endocannabinoid metabolism. *Chem. Biol.* 16, 744–753.
- Mackie, K., and Stella, N. (2006). Cannabinoid receptors and endocannabinoids: evidence for new players. *AAPS J.* 8, E298–E306.
- Marnett, L.J. (1992). Aspirin and the potential role of prostaglandins in colon cancer. *Cancer Res.* 52, 5575–5589.
- McGarry, J.D., and Brown, N.F. (1997). The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur. J. Biochem.* 244, 1–14.
- McMahon, B., and Kwaan, H.C. (2008). The plasminogen activator system and cancer. *Pathophysiol. Haemost. Thromb.* 36, 184–194.
- Menendez, J.A., and Lupu, R. (2007). Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat. Rev. Cancer* 7, 763–777.
- Mills, G.B., and Moolenaar, W.H. (2003). The emerging role of lysophosphatidic acid in cancer. *Nat. Rev. Cancer* 3, 582–591.
- Nomura, D.K., Blankman, J.L., Simon, G.M., Fujioka, K., Issa, R.S., Ward, A.M., Cravatt, B.F., and Casida, J.E. (2008). Activation of the endocannabinoid system by organophosphorus nerve agents. *Nat. Chem. Biol.* 4, 373–378.
- Patricelli, M.P., Giang, D.K., Stamp, L.M., and Burbaum, J.J. (2001). Direct visualization of serine hydrolase activities in complex proteomes using fluorescent active site-directed probes. *Proteomics* 1, 1067–1071.
- Prentki, M., and Madiraju, S.R. (2008). Glycerolipid metabolism and signaling in health and disease. *Endocr. Rev.* 29, 647–676.
- Przybytkowski, E., Joly, E., Nolan, C.J., Hardy, S., Francoeur, A.M., Langelier, Y., and Prentki, M. (2007). Upregulation of cellular triacylglycerol-free fatty

acid cycling by oleate is associated with long-term serum-free survival of human breast cancer cells. *Biochem. Cell Biol.* 85, 301–310.

Puustinen, P., Junttila, M.R., Vanhatupa, S., Sablina, A.A., Hector, M.E., Teittinen, K., Raheem, O., Ketola, K., Lin, S., Kast, J., et al. (2009). PME-1 protects extracellular signal-regulated kinase pathway activity from protein phosphatase 2A-mediated inactivation in human malignant glioma. *Cancer Res.* 69, 2870–2877.

Ren, J., Xiao, Y.J., Singh, L.S., Zhao, X., Zhao, Z., Feng, L., Rose, T.M., Prestwich, G.D., and Xu, Y. (2006). Lysophosphatidic acid is constitutively produced by human peritoneal mesothelial cells and enhances adhesion, migration, and invasion of ovarian cancer cells. *Cancer Res.* 66, 3006–3014.

Saghatelian, A., Trauger, S.A., Want, E.J., Hawkins, E.G., Siuzdak, G., and Cravatt, B.F. (2004). Assignment of endogenous substrates to enzymes by global metabolite profiling. *Biochemistry* 43, 14332–14339.

Sarnataro, D., Pisanti, S., Santoro, A., Gazzero, P., Malfitano, A.M., Laezza, C., and Bifulco, M. (2006). The cannabinoid CB1 receptor antagonist rimobant (SR141716) inhibits human breast cancer cell proliferation through a lipid raft-mediated mechanism. *Mol. Pharmacol.* 70, 1298–1306.

Seftor, E.A., Meltzer, P.S., Kirschmann, D.A., Pe'er, J., Maniotis, A.J., Trent, J.M., Folberg, R., and Hendrix, M.J. (2002). Molecular determinants of human uveal melanoma invasion and metastasis. *Clin. Exp. Metastasis* 19, 233–246.

Smith, C.A., Want, E.J., O'Maille, G., Abagyan, R., and Siuzdak, G. (2006). XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. *Anal. Chem.* 78, 779–787.

Stylli, S.S., Kaye, A.H., and Lock, P. (2008). Invadopodia: at the cutting edge of tumour invasion. *J. Clin. Neurosci.* 15, 725–737.

Vazquez-Martin, A., Colomer, R., Brunet, J., Lupu, R., and Menendez, J.A. (2008). Overexpression of fatty acid synthase gene activates HER1/HER2 tyrosine kinase receptors in human breast epithelial cells. *Cell Prolif.* 41, 59–85.

Wang, D., Wang, H., Ning, W., Backlund, M.G., Dey, S.K., and DuBois, R.N. (2008). Loss of cannabinoid receptor 1 accelerates intestinal tumor growth. *Cancer Res.* 68, 6468–6476.

Welch, D.R., Bisi, J.E., Miller, B.E., Conaway, D., Seftor, E.A., Yohem, K.H., Gilmore, L.B., Seftor, R.E., Nakajima, M., and Hendrix, M.J. (1991). Characterization of a highly invasive and spontaneously metastatic human malignant melanoma cell line. *Int. J. Cancer* 47, 227–237.

Wise, D.R., DeBerardinis, R.J., Mancuso, A., Sayed, N., Zhang, X.Y., Pfeiffer, H.K., Nissim, I., Daikhin, E., Yudkoff, M., McMahon, S.B., et al. (2008). Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proc. Natl. Acad. Sci. USA* 105, 18782–18787.

Yuan, T.L., and Cantley, L.C. (2008). PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 27, 5497–5510.

Zechner, R., Strauss, J.G., Haemmerle, G., Lass, A., and Zimmermann, R. (2005). Lipolysis: pathway under construction. *Curr. Opin. Lipidol.* 16, 333–340.

Zhao, Q., He, Z., Chen, N., Cho, Y.Y., Zhu, F., Lu, C., Ma, W.Y., Bode, A.M., and Dong, Z. (2005). 2-Arachidonoylglycerol stimulates activator protein-1-dependent transcriptional activity and enhances epidermal growth factor-induced cell transformation in JB6 P+ cells. *J. Biol. Chem.* 280, 26735–26742.

Zhou, W., Han, W.F., Landree, L.E., Thupari, J.N., Pinn, M.L., Billign, T., Kim, E.K., Vadlamudi, A., Medghalchi, S.M., El Meskini, R., et al. (2007). Fatty acid synthase inhibition activates AMP-activated protein kinase in SKOV3 human ovarian cancer cells. *Cancer Res.* 67, 2964–2971.

Zunder, E.R., Knight, Z.A., Houseman, B.T., Apsel, B., and Shokat, K.M. (2008). Discovery of drug-resistant and drug-sensitizing mutations in the oncogenic PI3K isoform p110 alpha. *Cancer Cell* 14, 180–192.