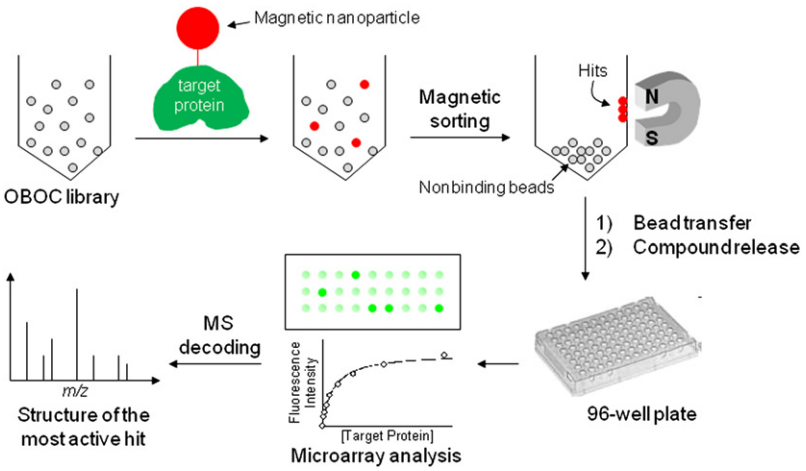

Figure 1. OBOC Library Screening by Magnetic Bead Sorting Coupled with Microarray Analysis

Library beads incubated with the target protein are rendered as a suspension in a tube. A magnet is placed next to the tube and the beads are allowed to settle. Negative beads settle to the bottom while the hits remain on the wall of the tube.

The recent developments described above have made OBOC library screening a much more efficient process. Indeed, it is now realistic for a single person to synthesize and screen a 100 million-member library and obtain the most active compound in a matter of weeks, without the need of any elaborate robotic systems. Although the methodologies have been demonstrated with peptide and peptoid libraries, they should be readily applicable to any compound class, as long as the compound structure can be decoded by using the sample from a single bead. For peptides and peptoids, compound decoding has become trivial due to the advent of several powerful mass spectrometry-based techniques (Paulick et al., 2006, Thakkar et al., 2009). For small molecule libraries of 10^4 or lower diversity, several innovative encoding techniques have been developed (Ohlmeyer et al., 1993, Song et al., 2003). For larger small-molecule libraries ($\geq 10^5$ diversity), hit identification remains a significant challenge.

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A New Age for MAGL

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Monoacylglycerol lipase (MAGL) has been known for its participation in triacylglycerol breakdown and endocannabinoid deactivation. Now Nomura et al. show that cancer cells can hijack MAGL to produce oncogenic lipid messengers. This exciting finding raises important pathophysiological questions.

Lipids are traditionally envisaged as energy fuels, membrane building blocks, and members of the steroid hormone family. However, most researchers rarely appreciate the high structural diversity and precise functional specialization of lipid metabolites. After the molecular characterization of a first generation of local (e.g., prostanoids) and intracellular (e.g., diacylglycerols [DAGs]) lipid messengers, a second group of lipids involved in crucial aspects of cell-to-cell communication has emerged during the past two decades. Examples include glycerol- and sphingoid base-backboned

lysophospholipids such as lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P), respectively, and new arachidonic acid bioactive derivatives such as endocannabinoids (Figure 1) (Piomelli et al., 2007; Takabe et al., 2008). These compounds are produced inside the cells, and their levels are tightly tuned by a complex balance between their synthesis and their degradation through a plethora of acyltransferases, lipases, lipid kinases, lipid phosphatases, and other enzymes. Once generated, these lipids can be released and act extracellularly in an autocrine/paracrine manner by engaging and activating G protein-coupled receptors (GPCRs), the largest superfamily of cell surface molecules involved in signal transduction (Dorsam and Gutkind, 2007; http://www.iuphar.org). Accruing evidence supports that this "inside-out" signaling by local lipid mediators may play a role in many human diseases, including cancer. For example, prostaglandin E_2 (PGE_2), S1P, and LPA induce cell proliferation, survival, and migration/invasion, thus favoring tumor growth, angiogenesis, and metastasis in animal models of cancer (Figure 1, in black) (Dorsam and Gutkind, 2007). In contrast, cannabinoid-receptor engagement usually evokes the opposite effects, at least under most cellular contexts (Figure 1, in red) (Guzmán, 2003).

Monoacylglycerol lipase (MAGL) has long been known for its participation in the breakdown of adipose triacylglycerol (TAG) stores, a key feature of metabolic homeostasis that, by providing tissues with free fatty acid (FFA) fuel, allows adaption to glucose deprivation periods (Figure 2A) (Tornqvist and Belfrage, 1976). However, the regulatory role of MAGL in this process seems to be negligible, as hydrolysis of the TAG *sn*-1 and *sn*-3 ester bonds by hormone-sensitive lipase (HSL) constitutes the pace-setting and hormonally-controlled step of lipolysis. After standing as a "dull" enzyme for many years, MAGL was "rediscovered" by Piomelli and coworkers, who showed that it plays a pivotal role in the deactivation of the endocannabinoid 2-arachidonoylglycerol (2-AG), a neuronal messenger that tunes the functionality and plasticity of many synapses (Figure 2B) (Dinh et al., 2002).

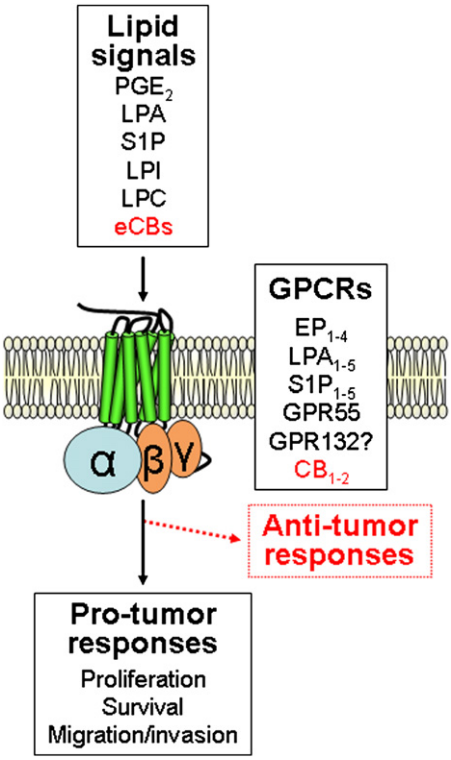

Figure 1. An Array of Local Lipid Messengers Controls Tumor Progression via GPCRs
Various lipid messengers are exported from cells and act in a local manner by engaging specific GPCRs. These signaling events usually evoke protumor responses such as cell proliferation, survival, and migration/invasion (in black). Cannabinoid-receptor engagement can exert antitumor effects (in red). eCB, endocannabinoid; LPC, lysophosphatidylcholine; LPI, lysophosphatidylinositol. Other abbreviations are in the text.

Now, Nomura et al. (2010) provide a new fascinating function for MAGL: the control of FA release from lipid stores in cancer cells, which allows them to produce the aforementioned lipid signals that promote tumor growth (Figure 2C).

Human tumors are highly heterogeneous in terms of nutrient supply and oxygenation, and develop progressive alterations in energetic and proliferative statuses as well as in the vascular network. Among dysregulated metabolic routes that correlate with malignancy, the shift from glucose oxidative metabolism to lactic acid-directed glycolysis (the Warburg effect) is so far the best documented example. The development of a lipogenic phenotype, characterized by enhanced FA synthesis de novo, also favors tumor growth in animal models and correlates with a poor prognosis in various types of human cancers (Menendez and Lupu, 2007). Enhanced lipogenesis may conceivably contribute to tumor growth by multiple mechanisms, including the generation of energy substrates, membrane building blocks, and oncogenic lipid precursors. As newly synthesized FAs are readily incorporated into cellular lipid stores, Nomura et al. (2010) reasoned that cancer cells should possess a complementary lipolytic pathway to liberate fatty acyl moieties from those reservoirs for signaling purposes. By using an elegant proteomic approach based on serine hydrolase activity-directed probes, they found that, among the dozens of serine hydrolases detected in their analyses, MAGL was consistently elevated in aggressive versus nonaggressive cancer cell lines, and accounted for the striking depletion of MAGs and enrichment of FFAs found in aggressive cells. Genetic or pharmacological blockade of MAGL in aggressive cells rendered them FFA-depleted and less malignant, while ectopic expression of MAGL in nonaggressive cells turned them FFA-enriched and more malignant. Of note, substantial experimental evidence was provided to favor the notion that MAGL supports the malignant properties of cancer cells by maintaining tonically elevated levels of FFAs, which, in turn, become mostly channeled to the production of various oncogenic lipid messengers, such as PGE_2 and LPA, rather than to fuel β-oxidation, sustain glycolysis, or generate membrane lipids, at least in the cancer cells tested (Figure 2C).

These hallmark observations by Nomura et al. (2010) raise fascinating conceptual and translational questions at the interface of the lipid metabolism and oncology fields. Let us mention three. First, the authors found that MAGL activity and FFA levels are elevated in high-grade versus benign human ovarian tumors. How do this newly discovered MAGL-FFA pathway and its associated secondary lipidomic signatures work in different types of cancer cells and their neighboring niches at different tumor initiation/progression stages? Plausible candidates to explore include colorectal and prostate tumors, in which MAGL could tune the levels of the protumorigenic lipids PGE_2 and LPA (Dorsam and

Gutkind, 2007), and perhaps of antitumorigenic endocannabinoids (Guzmán, 2003; Wang et al., 2008). Second, their study also unveiled that the low FFA levels and impaired growth of MAGL knocked-down cancer cells in xenografted mice can be rescued by feeding the animals a high-fat diet. What is the precise role of the MAGL-FFA connection in the crosstalk between obesity and tumorigenesis? Its possible link with dietary restriction and the oncogenic phosphatidylinositol 3-kinase pathway (Kalaany and Sabatini, 2009) could specifically be examined. Third, it was also shown that MAGL-silenced cancer cells display enhanced sensitivity to the antimigratory and antisurvival effects of an epidermal growth factor receptor tyrphostin. Could MAGL inhibitors render human tumors more responsive to the antineoplastic action of tyrosine kinase-targeted chemotherapeutic drugs? Although acute/pharmacological and sustained/genetic loss of MAGL function generated somewhat distinct metabolomic profiles in the cancer cells studied, the work by Nomura et al. (2010) turns MAGL into an unexpected and exciting new candidate for cancer therapy.

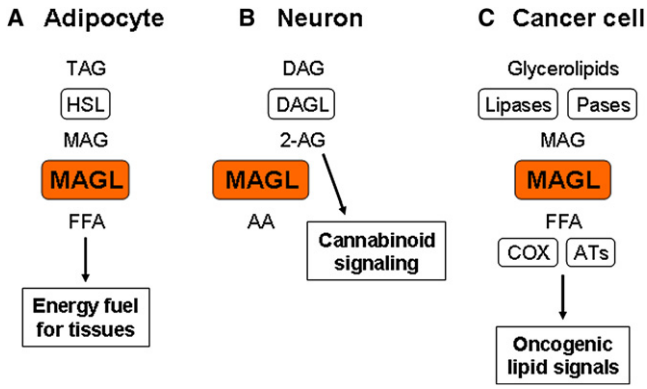

Figure 2. The Three Ages of MAGL

(A) MAGL catalyzes the final step of lipolysis in adipocytes, thus providing tissues with FFA fuel.

(B) MAGL deactivates 2-AG in neurons, thus tuning endocannabinoid signaling.

(C) MAGL controls FFA generation in cancer cells, thus allowing them to produce oncogenic lipid signals.

AA, arachidonic acid; AT, acyltransferase; COX, cylooxygenase; DAGL, *sn*-1 DAG lipase; Pase, lipid phosphatase. Other abbreviations are in the text.

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Mechanism-Based Neddylation Inhibitor

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Brownell et al. (2010) elucidate the mechanism of action of MLN4924, a NEDD8-activating enzyme inhibitor. MLN4924 requires the activity of the enzyme to generate a NEDD8-adenylate analog that potently and selectively shuts down this posttranslational modification system.

The covalent modification of proteins with ubiquitin (Ub) and ubiquitin-like proteins (UbLs) is widely appreciated as a major eukaryotic posttranslational regulatory mechanism. To date, nine classes of UbLs with varying degrees of identity to Ub have been identified in humans (Schulman and Harper, 2009). In addition to Ub's well appreciated role in promoting protein destruction by the 26S proteasome, Ub and UbLs have numerous other functions to rapidly and conditionally alter protein function, such as changing protein localization, regulating other posttranslational modifications, and modulating protein-protein interactions.

Ub and UbLs share similar enzymatic mechanisms for their conjugation onto target proteins. Their entry into a conjugation pathway first requires ATP-dependent activation through the formation of an adenylate intermediate in the nucleotide binding pocket of their cognate E1 (activating enzyme) (Ciechanover et al., 1981; Haas et al., 1982). After this occurs, the activated molecule is transferred onto the catalytic cysteine of E1, an acceptor

