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Time for lipid cell biology

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It is an exciting time for lipid metabolism and membrane cell biologists as technological progress has increased our ability to study lipids in cells. We asked leaders studying lipid cell biology from different perspectives to share what questions they are most interested in and what tools they believe the field is currently lacking.

Xiao-Wei Chen: The MATH of cholesterol and phospholipids

Lipids, although often linked to energy stores and obesity, are indispensable building blocks of cellular structures. Phospholipids and cholesterol, in particular, form biological membranes that encapsulate and define cells and organelles. Unlike neutral lipids such as triglycerides, cells cannot store large amounts of amphipathic lipids. Their timely production and distribution, both within and across cells, could therefore be a matter of life-or-death.

Cholesterol offers a prime example of a co-ordinated homeostasis in metabolism and transport of key membrane-building lipids. Synthesized at the endoplasmic reticulum, cholesterol is efficiently distributed to other membrane organelles, constituting a distinct concentration gradient in which the endoplasmic reticulum membrane contains the lowest cholesterol content. In specialized cells such as hepatocytes and enterocytes, cholesterol traverses the endoplasmic reticulum membrane to be packed into lipoproteins for secretion. Conversely, when cholesterol is taken up via lipoprotein endocytosis, it is shuttled across lysosome membranes by NPC1 and NPC2, and eventually reaches the endoplasmic reticulum membrane to signal a feedback loop to halt further cholesterol production.

Despite these major advances, fundamental questions remain concerning the spatio-temporal dynamics of cholesterol in cells, partly due to limitations in tools available to faithfully track the molecule. These include how cells transport and distribute cholesterol after its biosynthesis in the endoplasmic reticulum, or how endocytosed cholesterol reaches and becomes incorporated into the endoplasmic reticulum membrane to initiate a metabolic signal. Moreover, the questions become more challenging when getting to the nanometre scales, either across or along the membrane. The precise mechanisms by which cholesterol efficiently traverses bilayer membranes, as well as the function of cholesterol-rich membrane nanodomains await further elucidation, especially at the endoplasmic reticulum where cholesterol content is low. Lastly, one may envision that such co-ordinated control in the metabolism and transport homeostasis (MATH) may extend to phospholipids and other amphipathic lipids. Understanding such MATH is vital, considering the number of mortalities and morbidities caused by lipid-driven diseases including cardiovascular and metabolic disorders.

Anthony S. Don: Advances in the application of lipidomics to reveal the aetiology of dementia

This is an exciting time to work in the field of lipid cell biology, as developments in instrumentation and analytical power will soon enable us to study lipid metabolism with single-cell resolution. Driven by advances in the speed, accuracy and sensitivity of mass spectrometry systems, the field of lipidomics has evolved over the past two decades to the point that we can now determine the precise structures for hundreds to thousands of lipids in any given sample and apply pulse–chase experiments with stable isotopes to track lipid metabolism in multicellular culture systems or living organisms. In parallel, imaging mass spectrometry now permits the resolution of lipid composition in the low micron range, such that single-cell lipidomic analysis in situ is becoming a reality.

One of many biomedical research areas in which this will have tremendous impact is in understanding the aetiology of dementia. The brain is one of the most lipid-rich organs and genomic studies tell us in no uncertain terms that gene variants affecting lipid metabolism are the primary determinants of genetic risk for Alzheimer's disease, vascular dementia and Parkinson's disease. Despite this, there has been very little research on the relationship between these genetic risk factors and brain lipid metabolism. Where lipidomic profiling has been applied to tissue samples, it has thus far failed to convincingly establish how these genetic risk factors combine with ageing to affect brain lipid homeostasis and promote neurodegeneration.

Part of the solution may be to move from cross-sectional lipidomic profiling that measures lipid levels at a snapshot in time to flux experiments that empower us to determine how gene and environment affect lipid synthesis and turnover. Another part of the solution lies in the power of spatial or single-cell technologies to unravel the contribution of specialized cell types to lipid synthesis, transport, metabolism and signalling. Combining the two will give us the analytical power to solve complex questions such as the aetiology of Alzheimer's disease.

Maria Fedorova: Evolving tools for functional studies of lipids

With the development of high-throughput and sensitive omics technologies, our knowledge on lipid metabolism has increased substantially. Modern mass spectrometry-based lipidomics is able to annotate several hundreds of lipid species and estimate their concentrations. Despite the wealth of molecular information, functional annotation of lipidomics data remains challenging. Lipids rarely function at the level of single species but rather in a collective manner as supramolecular lipid assemblies, represented by a set of specific molecular species at a given relative abundance. Such lipid

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assemblies, rather than individual lipid species, represent the true functional units that carry out a variety of cellular functions. And although, conceptually, it is well appreciated that lipids act collectively (for example, lipid microdomains in biological membranes), so far there are no practical means to attribute these 'lipid collectives' to functional outcomes from lipidomics datasets. Development of bioinformatics tools and application of novel machine learning algorithms will potentially help to define the composition of functional lipid assemblies and facilitate functional annotation of lipidomics datasets.

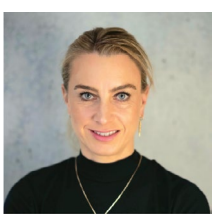
Another challenge in studying cell biology using lipidomics readouts is that we still have rather limited knowledge on cell type specificity and heterogeneity in terms of lipid metabolism. However, it is crucial for the understanding of metabolic plasticity and discriminating between adaptative and maladaptive responses. Spatial biology including mass spectrometry imaging and single-cell lipidomics will no doubt provide a wealth of information on the lipidome composition of different cell types in their native environment, but will also allow the natural heterogeneity of cellular lipid metabolism to be addressed. Furthermore, application of lipid flux analysis using stable isotopes and/or biorthogonal labelling technologies (for example, based on click chemistry) already delivers valuable information on the close connectivity and temporal regulation of lipid metabolic pathways. Spatial and temporal resolution of lipid metabolism at molecular level will open up new dimensions in lipid cell biology research.

Takeshi Harayama: Structure–function relationship of membrane lipids

Lipids are extremely diverse in structures, with different backbones, head groups and tails that vary in lengths, double-bond numbers, double-bond positions and hydroxylations. Lipidomics revealed that these lipids are used to generate membranes that differ in composition at multiple scales: organisms, cell types, organelles, membrane leaflets and distance from membrane proteins. However, besides lipids that are receptor ligands or those that recruit lipid-binding proteins to membranes, the mechanisms by which lipid structures and lipid-collective behaviours affect biological processes are rarely elucidated. Therefore, a key question is to establish the structure–function relationship of the numerous lipids present in membranes, which will lead to better interpretations of lipidome changes in various diseases and the corresponding causality.

Mechanisms for how the structures and dynamics of lipid tails affect cellular processes are particularly unclear. For example, by definition, omega-3 and omega-6 polyunsaturated fatty acids differ only in the positions of double bonds, yet their biological roles clearly differ. It is still unclear whether their functional differences are mediated by their effects on membrane physical properties or membrane-associated protein functions. Also, there is ample evidence that lipid tails contribute to the generation of lateral heterogeneities (or domains) in membranes, for example, in yeast vacuoles, but mechanisms for how heterogeneities emerge are often unclear. It is likely that they result from a complex interplay between lipids, membrane-associated proteins, the cytoskeleton and other molecules that we still ignore, but we do not know in which order individual factors come into play. Elucidating the roles of lipids in heterogeneity formation will reveal how lipid structures affect membrane trafficking, signalling, organelle functions and many other processes.

Technically, we need better tools to analyse lipid localization, manipulate cellular lipid compositions and test how lipids affect the activities of membrane-associated proteins. In particular, tools for unbiased identification of proteins with altered dynamics in distinct cellular lipid environments will be of outstanding interest. Obviously, this asks for a combination of techniques, such as chemical biology, genetic engineering, analytical chemistry and computational biology. This need for interdisciplinarity is what makes lipid research challenging but also exciting.



Natalie Krahmer: Unlocking the mysteries of cellular fat storage

Lipid droplets (LDs), once seen as simple fat stores, are now recognized as key players in metabolic regulation. Over the past decade, research has uncovered the machinery behind LD formation and degradation, revealed the LD proteome and shown how proteins are targeted to LDs. Despite these advances, important questions remain, presenting opportunities for further investigation, especially in relation to diseases.

A challenge is understanding the roles of LD-associated proteins, as, for many of them, their function is still poorly understood. For instance, the physiological functions of PNPLA312 and HSD17B1313, well-established risk factors for fatty liver disease, remain unclear. Clarifying their roles could lead to treatment strategies.

Another important aspect is the interaction of LDs with other organelles. These interactions are key for energy homeostasis but remain unclear. LDs attached to mitochondria reportedly have distinct proteomes and functions, raising important questions about how these contacts are established and whether and how they control organelle compositions and functions.

LDs show considerable heterogeneity across tissues and cell types. For instance, hepatic stellate cells store retinyl esters, while adrenal LDs primarily store cholesteryl esters with unique crystalline structures, raising questions about how lipid composition affects the LD proteome and function. Even within the same cell type in tissues, lipid storage can vary greatly among cells, as it was recently shown for adipose tissue, and LDs within a single cell can differ in size, lipid content and proteome. Understanding this variability and its physiological importance is crucial.

Beyond lipid storage, LDs have poorly understood roles in signalling and transcription regulation. In adipocytes, LD size affects leptin secretion, vital for maintaining energy balance by signalling the state of fat stores to the brain. However, how LD size is sensed and regulates leptin expression remains a mystery. Addressing these unanswered questions could unlock new insights into metabolic regulation and disease.

Shigekazu Nagata: Exposure of phosphatidylserine on the cell surface

Biological membranes consist of lipid bilayers that not only separate the extracellular and cytoplasmic spaces but also compartmentalize organelles. The four major phospholipids — phosphatidylserine (PtdSer), phosphatidylcholine, phosphatidylethanolamine and sphingomyelin — are asymmetrically distributed across the leaflets of the membrane. PtdSer is typically confined to the inner leaflet by flippases, enzymes that actively and specifically transport it inward.

During certain biological processes, PtdSer is externalized by scramblases, which randomly redistribute phospholipids across the bilayer. This externalization serves as a crucial signal in various cellular contexts. For example, during apoptosis, cells expose PtdSer on their surface, signalling macrophages to engulf them. While the mechanisms by which apoptotic cells expose PtdSer and how it is recognized by macrophage receptors are well understood, the processes governing PtdSer exposure in senescent cells remain elusive. In the small intestine, epithelial cells undergoing turnover expose PtdSer to signal their removal, and during neural development, PtdSer exposure precedes the pruning of unnecessary synapses. However, these processes are not yet fully elucidated.

PtdSer also plays a key part in cell fusion events, such as those occurring in osteoclasts, myotubes and syncytiotrophoblasts, where its transient exposure facilitates membrane fusion. Despite this, the exact molecular mechanisms of PtdSer exposure and its specific role in these fusion events remain unclear.

Moreover, activated platelets expose PtdSer and release PtdSer-exposing microparticles, which serve as scaffolds for clotting factors, facilitating their activation. Although the exposure of PtdSer and the production of these particles in platelets are well studied, the mechanisms underlying the generation of other PtdSer-exposing cells and particles — such as activated T cells, milk fat globules in mammary epithelial cells, exosomes and enveloped viruses — are less well understood. Unlike apoptotic cells, activated platelets and T cells are not engulfed by macrophages. The hypothesis that irreversibly exposed PtdSer, rather than reversibly exposed PtdSer, serves as an 'eat me' signal requires further investigation.

Priyanka Narayan: Lipid biology affects neurodegenerative disease

Lipids function as both structural and signalling molecules in all cell types. The brain is one of the most lipid-rich organs in the human body. Consequently, disruptions to lipid biology are central to several brain diseases.

Lipid accumulation is a pathological hallmark of common neurodegenerative diseases such as late-onset Alzheimer's disease and Parkinson's disease. Genetic and genomic studies have identified polymorphisms in lipid metabolism genes as strong risk factors for Alzheimer's disease (for example, *APOE*, *PLCG2* and *ABCA7*), Parkinson's disease (for example, *GBA*) and other late-onset neurodegenerative diseases. For many years the neurodegeneration field focused on protein aggregate-centric biology. However, we now know that changes to lipid metabolism affect both cell autonomous and cell non-autonomous phenotypes central to neurodegenerative disease biology such as amyloid-beta uptake, tau phosphorylation, neuronal communication, lysosomal function, alpha-synuclein aggregation and microglial inflammation.

Despite the strong genetic and functional associations between lipid metabolism and late-onset neurodegenerative disease risk, the underlying molecular mechanisms remain unresolved. Advances in mass spectrometry-based methodology and cell biology assays to characterize and target cellular lipid metabolism have enabled the quantitative study of lipid biology in the context of cellular and animal models. Induced pluripotent stem cell-derived tissues allow us to study common and cell type-specific phenomena in human brain cell types.

Now, our challenge is to use these tools to characterize how lipid metabolism is perturbed in different brain cell types in the contexts of neurodegenerative disease risk. We are poised to unearth both the causes and the functional consequences of key lipid metabolic disruptions. And with this knowledge, we can aim to modulate metabolism to mitigate disease risk.

Genetic studies in Alzheimer's disease have also identified protective polymorphisms in lipid metabolism genes such as *APOE* and *PLGC2*. A new frontier in Alzheimer's disease research is to understand whether these protective polymorphisms confer resilience to disease by reshaping cellular lipid metabolism. By focusing on resilience biology rather than risk, this approach can illuminate new protective or preventative strategies for these devastating diseases.

Dequina Nicholas: A perspective shift in immunometabolism from lipid metabolism to lipid presentation

The field of immunometabolism has explored how lipid metabolic pathways influence adaptive immune cell function. However, lipids can also trigger adaptive immune activation through classical mechanisms, including B cell receptor engagement and antigen presentation to T cells. In the 1990s, it was discovered that CD1 molecules (class I, CD1a, CD1b and CD1c; and class II, CD1d and CD1e) are analogous to the major histocompatibility complex but present lipids. Despite this discovery, the effect of lipid antigen presentation in human health remains underexplored. The role of CD1d in bacterial activation of invariant natural killer T cells is an exception.

The absence of literature on this topic is driven by (1) inadequate animal models (mice lack class I CD1) and (2) technical challenges in measuring lipids in aqueous systems. These challenges prevent exciting questions from being asked, including how the immune system senses dietary lipids and the homeostatic role of adaptive immune responses to endogenous lipids. Immune cell function shifts with diet and even between the fed and fasted state, and it is likely that gut immune cells programme systemic lipid metabolism. Transgenic mice expressing class I CD1, used to demonstrate a role for CD1a in skin inflammation, are a practical model for understanding antigen-specific immune responses to dietary lipids. Pigs, which express class I CD1 molecules and are metabolically similar to humans, are increasingly viable models with an expanding catalogue of flow cytometry antibodies.

Finally, advancing the field requires adapting approaches for protein interactions to the study of lipid immune reactivity. ELISA, flow cytometry and cell sorting rely on aqueous solutions and soluble antibodies, and thus it is difficult to label cells with lipids that interact with membrane proteins with any degree of certainty or specificity. Interdisciplinary efforts to develop tools to allow identification, isolation and manipulation of lipid-specific immune cells would create the potential to expand immunometabolism from lipid metabolic pathways to lipid sensing and systemic lipid regulation.

Sara M. Nowinski: Understanding the role of mtFAS

Contrary to their well-known role in fatty acid oxidation, mitochondria also build fatty acids in the matrix via the mitochondrial fatty acid synthesis (mtFAS) pathway. mtFAS is essential for oxidative function and well-conserved across species: from yeast, where mtFAS is required for respiratory growth, to mammals, where loss of the pathway is embryonic

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lethal in mice. The pathway uses acetyl-CoA to build the eight-carbon lipid lipoic acid, a requisite co-factor for mitochondrial dehydrogenases (including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase). mtFAS also builds longer fatty acid products, which facilitate physical interactions with the acyl carrier protein on which they are built. These interactions functionally support the FeS cluster biogenesis complex, and several assembly factors for the electron transport chain. However, the exact identity (or identities) and ultimate cellular destination(s) of these longer FA products remain unknown. Moreover, despite recent advances in our understanding of how mtFAS promotes oxidative mitochondrial metabolism, the regulation of the pathway, function in diverse tissue settings and role(s) in disease are largely unexplored.

The evolutionary persistence and essentiality of mtFAS is a teleological mystery. Mitochondria take up lipids for many other purposes: they import acyl-carnitines for fatty acid oxidation and perform key steps in the biosynthesis and or metabolism of phospholipids, cholesterol, steroid hormones, co-enzyme Q and Vitamin D. Why, then, can mitochondria not scavenge abundant mitochondrial fatty acids or exogenous lipoic acid to replace those built de novo by mtFAS? One potential reason is that mtFAS ties many facets of oxidative metabolism (TCA enzyme activity, FeS clusters and electron transport chain assembly) back to the ultimate substrate for catabolism: acetyl-CoA. As a result, the pathway is primely placed to serve as a central regulatory node in mitochondria, supporting increased oxidative capacity during times of nutrient excess or co-ordinating its shut-off when fuels are scarce. This model is supported by data in yeast, where knock-out of the mitochondrial pyruvate carrier reduces mtFAS activity but is yet to be demonstrated in mammalian systems. Future studies aimed at understanding how metabolite availability affects pathway activity in mammalian cells will be key to uncovering its role in the nutrient-sensitive regulation of mitochondrial function.

Yasunori Saheki: Control of intracellular lipid distribution via non-vesicular lipid transport at membrane contact sites

Cells maintain an uneven distribution of membrane lipids across different cellular compartments, which is important for their integrity, identity and functions. While vesicular transport, which relies on membrane budding and fusion, plays a key part in bulk transport of lipids from one compartment to another, this process is often slow and non-selective. Additionally, mitochondria are not connected to the vesicular transport system, making it insufficient for delivering lipids to this essential organelle.

Over the years, numerous studies have revealed the crucial role of non-vesicular transport in more rapid and selective lipid transport and exchange. In the past decade, our understanding of this process has grown exponentially. We now know that non-vesicular transport is often mediated by lipid transfer proteins (LTPs) at membrane contact sites formed between organelles without membrane fusion, contributing to the control of intracellular lipid distribution. In vitro reconstitution of these reactions, supported by analyses of protein–lipid interactions using structural and biochemical approaches, as well as molecular dynamic simulations, have been instrumental in advancing our understanding of the lipid transfer kinetics and ligand selectivity of LTPs. Likewise, advanced microscopy techniques, such as high-resolution live-cell imaging and super-resolution microscopy combined with novel lipid biosensors and probes, have provided valuable insights into the dynamic localization of LTPs and their roles in intracellular lipid distribution.

Despite these advances, several critical questions remain unanswered. Efforts are needed to better understand the importance of non-vesicular lipid transport and its regulation across different cellular and developmental contexts. Investigating the functional redundancy of LTPs, especially using genetically tractable organisms such as *Caenorhabditis elegans* and *Drosophila melanogaster*, is also crucial. Human genetic studies have shown a strong link between the dysfunction of LTPs and various human disorders. However, the physiological impact of LTPs in health and disease is still poorly understood. For example, while there is a well-established association between dysregulated lipid homeostasis and neurodegenerative disorders, including Parkinson's disease and Alzheimer's disease, we still lack a complete understanding of how LTPs function in neurons and other brain cell types, such as astrocytes, oligodendrocytes and microglia, to maintain lipid homeostasis in the brain. As we deepen our understanding of the physiological roles of LTPs, developing strategies to manipulate their activities will be essential. Such approaches could harness the lipid transfer properties of LTPs to restore lipid homeostasis in human disorders, potentially mitigating or even reversing disease progression.

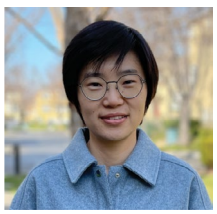
Clay F. Semenkovich: Lipid modification of proteins and disease

Lipid cell biology, similar to great art, is associated with a sense of uncertainty. A Jackson Pollock action painting evokes continual motion driven by an uncanny perception of order, resembling the frenetic movement, fusion and remodelling of organelles and membranes of a live cell. Exocytosis, non-canonical secretion, dynamic functions at membrane contact sites, active transport and other processes require proteins that adapt to lipid environments that constantly change and differ between subcellular domains. One adaptation is lipid modification of proteins, increasingly recognized as relevant to disease.

S-palmitoylation, the most common form of lipidation, is reversible and affects >10% of human proteins. It involves the addition of palmitate (16:0) to cysteine residues allowing normal intracellular trafficking by promoting membrane association and protein–protein interactions. More than 20 zDHHC acyltransferases create a thioester bond to mark proteins with palmitate (often derived from de novo lipogenesis) and a small group of depalmitoylases (APT1, APT 2, PPT1, PPT2, and αβ hydrolase domain enzymes) erase the mark. In multiple tissues, including neurons, endothelial cells and pancreatic islets, palmitoylation co-ordinates other post-translational modifications such as phosphorylation, nitrosylation, acetylation and ubiquitination. Altered palmitoylation is implicated in neurodegeneration (approximately 50% of synaptic proteins are palmitoylated), vasculopathy (eNOS and R-RAS), diabetes (post-Golgi recycling proteins and mitochondrial proteins), cancer (PD-L1, RAS isoforms and EGFR), inflammatory disorders (STING, gasdermin and NLRP3 inflammasome), viral infections (SARS-CoV-2 spike protein) and many other diseases.

Given the functional redundancy for marking and erasing palmitate on proteins, we need a better understanding of how palmitoylation is regulated. Do cells use metabolites, phase

separation, pH, reactive oxygen species, intracellular calcium, membrane tension or other factors to alter palmitoylation status? There are substantial sex differences in biological responses of palmitoylated proteins. Does S-palmitoylation underlie sex-based differences in inflammatory disorders such as autoimmunity and atherosclerosis? Palmitoylation detection by current chemical approaches and mass spectrometry is cumbersome, and the generation of antibodies to palmitoylated peptides has been disappointing. New analytical and chemical techniques have the potential to establish mechanistic paradigms to resolve the sense of uncertainty that limits the application of palmitoylation to disease.



Xiaoai Zhao: Uncovering the functional diversity of lipids in aging and diseases

By way of introduction, lipids are often referred to as one of the 'cellular building blocks'. As much as this description highlights the crucial contribution of lipids in biological systems, this depiction does not capture the dynamic functional roles of lipids. Research in the past decades has begun to shed light on the intricate connections between lipid metabolism, ageing and diseases. However, the extent of the contribution of lipids in physiology and diseases has only started to emerge. There are a great number of questions and challenges ahead regarding lipids in cell biology, the answers to which could give us insight into targeting lipids as the next frontier in combatting ageing and diseases.

Mammals have one of the most complex lipidomes. The chemical diversity of lipid species has been increasingly appreciated, however the implications of what distinct chemical properties can confer in biological processes remains largely unexplored. Phospholipid remodelling enzymes directly contribute to the diversity of phospholipids, by modifying the acyl-chain composition of de novo synthesized lipids. Functional studies on this class of enzymes have uncovered the consequences of perturbed lipid side chain composition in pathologies including non-alcoholic fatty liver disease, atherosclerosis and cancer. Ongoing and future studies aimed at elucidating the mechanistic regulation of lipid composition on their biophysical properties, cell signalling and lipid–protein interactions will provide valuable insight.

At a cellular level, many abundant glycerophospholipid and sphingolipid species can be found in different organelles and compartments across the cell. However, how these lipid classes are regulated in a concerted manner between very different subcellular environments is not well understood. For instance, it has been suggested in mouse studies that ageing is associated with increased abundance of polyunsaturated fatty acid-containing glycerophospholipids across multiple tissues. But are these composition changes occurring to the same degree in different organelles? If not, what is the role of organelle-specific regulation in shaping the trajectory of ageing and diseases? Answers to these questions may guide us toward harnessing the complexity of the mammalian lipidome as a new avenue in understanding the fundamental biology of human ageing and diseases.

Yilong Zou: Lipotypes and cancer cell behaviour, a systems biology view

One open question in the field is how the lipidome, together with components of the central dogma, regulates cell behaviour, such as malignant transformation. A worthwhile approach is establishing a 'genotype–lipotype–phenotype' connection at single-cell resolution. The lipotype can be defined as the collection of cellular lipidome compositions, and the metabolic circuits mediating chemical inter-conversions. Recent advances in multi-omics technologies reinforced the notion that various tissue- and cell-types differ in lipidome compositions, yet the precise contributions of lipidome diversity to cell functions are largely unclear.

In human tumours, malignant clones often exhibit complex behaviours during different stages of disease progression, including proliferation, immune editing and evasion, invasion and metastasis, cell cycle arrest and dormancy, as well as adaptation to therapeutic stress. How does the lipidome support each of these functionalities, and how plastic does the lipidome have to be to support cell-state transitions? For instance, tumour cells surviving in primary carcinomas, ascites and metastatic sites exhibit different lipidomic profiles, which modulate tumour metastatic potential at multiple steps. We envision systematic characterization of the lipotypes and cancer cell behaviour may reveal therapeutically exploitable vulnerabilities in cancer.

Meanwhile, advances in the following technologies will accelerate discoveries: development of sensitive and selective probes that report the physico-chemical characteristics of individual and packed lipids, to be combined with super-resolution imaging to record spatio-temporal dynamics of lipid moieties; advanced structural biology tools to visualize lipid macromolecule assemblies in cells; and single-cell lipidomics and spatial lipidomics at single-cell resolution to report lipidome composition in cells and tissues in situ. As an extension, co-detecting lipids, transcripts and/or proteins with spatial co-ordinates in the same samples will be particularly informative. Furthermore, 'functional' single-cell lipidomics, which awaits matured mass spectrometry barcoding strategies to integrate high-throughput genetic perturbations with lipidomics, will accelerate the 'correlation' to 'causation' transition; as multi-dimensional lipidomics data enrich, machine-learning-based approaches to identify connections between lipotypes and other cellular features will be feasible. Enabled by these data, example explorations include identifying lipid groups co-regulated in diverse contexts to uncover principles underlying concerted regulations and depicting a comprehensive protein–lipid interactome at atomic resolution. Overall, integrating lipid cell biology with systems biology tools will help us to establish a more holistic view of lipid functions in cell physiology.

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