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ALZHEIMER'S DISEASE THERAPEUTICS – ARE WE MAKING PROGRESS TOWARDS A DISEASE-MODIFYING TREATMENT FOR PATIENTS? HIGHLIGHTS FROM THE SOCIETY FOR MEDICINES RESEARCH SYMPOSIUM, HELD JUNE 20, 2016

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SUMMARY

Alzheimer's disease is arguably the largest healthcare issue of our time, with over 45 million people currently diagnosed with dementia worldwide. With the single biggest risk factor being age, this number is only going to increase as our populations' age. Alzheimer's disease pathology is highly complex, but is believed to be principally the result of an inter-play between the toxic proteins β-amyloid and tau and driven by several genetic and environmental risk factors. Current treatments focus on treating cognitive and behavioral symptoms but have only modest effects and duration of efficacy. To discuss these topics,

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a 1-day symposium was held in Brussels to look at the progress that the field has made over recent years in attempting to target different aspects and stages of Alzheimer's disease pathology and what lessons have been learned. It also looked at the current biological mechanisms under clinical and preclinical evaluation, together with exploring how new and developing genetic insights into the disease are directing us to novel mechanisms for potential new treatment options and to address the critical question: "Are we making progress towards a disease-modifying treatment for patients?"

THE LANDSCAPE FOR ALZHEIMER'S DISEASE THERAPEUTICS: CHALLENGES AND OPPORTUNITIES

Dr. Simon Ridley, Director of Research, Alzheimer's Research UK, provided the opening lecture on the current landscape of the dementia research environment, initiatives/investments and our progress towards therapeutic interventions to change the quality of life of patients and carers living with Alzheimer's disease (AD). Although two-thirds of dementia cases are over the age of 80 and classified as AD, there are multiple and varied underlying pathologies at play. This leads to AD being a complex illness with varied neuropathology and subsequent comorbidities, leading to difficult diagnosis and treatment. Dr. Ridley highlighted the gaps that still exist in our understanding of the disease. These included i) understanding the mechanisms of synaptic dysfunction, cellular degeneration and ultimately neuronal death; ii) selective vulnerability/resilience (stemming from genetic backgrounds and concomant diseases, e.g., diabetes) on neurones and their networks and; iii) roles of the vasculature and systemic immunity. He also highlighted the need to move beyond drug targets focused around the β-amyloid systems for future therapeutic intervention strategies. Technology gaps also still exist in the preclinical arena particularly around cellular and animal models that have construct validity for the disease pathology, while

understanding their limitations around predictive use. Tools for clinical stratification of volunteers, target engagement and measures of efficacy are still lacking. Biomarkers for diagnosis, linking diagnosis with pathology and tracking clinical therapeutic efficacy are also still needed, although some progress has been made. However therapeutic intervention points for disease-modifying approaches that are currently undergoing clinical testing will be relatively late in the disease process, therefore the impact on the patient well-being and symptoms is unknown, and thus it is likely there will still be a need for improved symptomatic treatments alongside the disease delaying approaches.

Dr. Ridley went on to describe how the dementia community has been galvanized to work together following the G8 dementia summit declaration to identify a disease-modifying therapy for dementia by 2025. He highlighted the enhanced funding and the need to build on innovation originating from academic research and also the strengths and challenges of these approaches (e.g., specialist knowledge and expertise vs. mismatch in expectations and appropriate schemes to incentivize basic research). As part of these, a number of examples were shown such as Innovative Medicines Initiative EU funding (e.g., European Prevention of Alzheimer's Disease Consortium [IMI-EPAD; www. ep-ad.org] and European Medical Information Framework [IMI-EMIF]; Medical Research Council funded Dementias Platform UK [DPUK; www.dementiasplatform.uk] and UK Dementia Research Institute; as well as ARUK's own Drug Discovery Alliance [at the Universities of Oxford, Cambridge and University College London]) and partnership initiatives to support drug discovery efforts such as The Dementia Consortium (www.dementiacopnsortium.org) and the Dementia Discovery Fund (www.theddfund.com).

Dr. Ridley finished by highlighting that we should not lose sight of the aim of this research, which is to ultimately improve the quality of life of patients and carers living with AD.

ADVANCES WITH ANTI-AMYLOID THERAPIES IN DEVELOPMENT FOR AD

Dr. Samantha Budd Haeberlein, VP Alzheimer's Discovery and Development, Biogen Idec, provided an insight into the progress we have made in the development of amyloid-focused therapeutics. The terrible nature of AD as a disease was highlighted, with the statistics suggesting that one patient is diagnosed every 67 seconds, and 5.4 million patients are currently diagnosed in the U.S. alone; a number which is expected to rise to 13.8 million by 2050 (1). In addition to this human cost, the implications to the financial status of our world's health systems are also going to be huge, with an estimated cost of USD 200 billion in the U.S. alone (2). Our developing understanding of the pathophysiology of AD has taken a considerable amount of time since Alois Alzheimer's first observations of plaques and tangles (3) through to the first description of the amyloid hypothesis (4). However, we now have a number of viable intervention strategies, from small-molecule inhibitors of the synthetic enzymes such as β-secretase (BACE) through to antibody approaches for removal of various forms of amyloid oligomers/fibrils/plaques. The reasons for this long timeline were outlined: finding the optimal drug, challenges of animal models, identifying the appropriate patients and best clinical endpoints and assessments. Two examples of BACE-targeted drugs that failed in the clinic were used to illustrate these points.

Firstly, semagacestat, which failed its phase III study due to low target engagement in the CNS, likely due to the dose-limiting safety issues related to poor selectivity over notch signaling pathways; and Flurizan (tarenflurbil) which also failed its phase III trial due to low CNS exposure of a relatively low potency molecule leading to limited enzyme inhibition *in situ*. Dr. Budd went on to describe the more successful development of the BACE inhibitor AZD-3293 (5). This molecule overcame many of the limitations of previous BACE inhibitors with high target affinity (K_i 0.4 nM) and cellular potency (IC_{50} ~0.1 to 0.6 nM). This molecule had good brain penetration and produced dose-related decreases in brain and CSF Aβ40 across multiple preclinical species as well as a sustained decrease in plasma and CSF Aβ42 in volunteers and patients (ID_{50} ~15 mg and a maximum decrease of ~90% in CSF Aβ42 at 150 mg). This molecule is currently in phase III. Finally the humanized monoclonal antibody selective for aggregated forms of β-amyloid, including soluble oligomers and insoluble fibrils, aducanumab was described. In the Tg2576 mouse model of amyloid pathology this antibody produced dose-related decreases in Aβ- and microglial-mediated phagocytosis of plaques. In the phase Ib clinical study, aducanamab produced dose- and time-related (1-10 mg/kg at 6 and 12 months) decreases in amyloid positron emission tomography (PET) imaging signals. Interestingly, using Clinical Dementia Rating Sum of Boxes (CDR-SB), as an exploratory measure, and Mini-Mental State Examination (MMSE) measures of cognition, researchers found that aducanumab produced a significant effect at the 10 mg/kg, and 3 and 10 mg/kg doses, respectively. Moreover, the CDR-SB measure correlated with the decreases seen with PET amyloid imaging at 52 weeks of treatment. The main safety finding was ARIA-E (amyloid-related imaging abnormalities - vasogenic edema), which was suggested to be monitorable and manageable. This antibody has proceeded to phase III testing. Dr. Budd concluded by highlighting the progress we have made in our ability to assess and test amyloid-targeted therapeutics using modern biomarker approaches. Despite the fact that multiple challenges still remain in patient identification and testing, since AD manifests long before symptoms, these are nonetheless exciting times for AD drug development.

NEW GENETIC DISCOVERIES HIGHLIGHT NOVEL DISEASE MECHANISMS IN AD

Professor Julie Williams, MRC Centre for Neuropsychiatric Genetics & Genomics, University of Cardiff, gave a summary of where the genetic analysis of Alzheimer's has taken us in terms of our knowledge of genetic vulnerabilities and the biological pathways implicated in the development of AD. Early susceptibility genes within familial AD populations, i.e., amyloid precursor protein (APP), presenilin 1 and 2, led to the formulation of the amyloid hypothesis (4). The largest risk factor/susceptibility gene for late–onset AD (LOAD) is ApoE, discovered some 17 years ago, and this remains the highest risk factor in the general population. In 2009 Professor Williams, as part of a large network of collaborators, identified clusterin, complement factor 1 and PICALM (phosphatidylinositol-binding clathrin assembly protein) from large patient number genome-wide association study (GWAS) studies (6, 7). More recently there have been major collaborations, such as the International Genomics Alzheimer's Project (IGAP), which have taken genetic analysis data from many tens of thousands of patients and identified 25, both rare and

common, gene loci which are associated with a vulnerability to AD (8). These loci are not, however, randomly distributed and biological pathway analysis shows significant evidence for the clustering of these genetic signals around key biological processes, i.e., innate immunity, cholesterol transport, ubiquitination and endocytosis. Professor Williams suggested that the search for new therapeutic targets should focus around these biological pathways and functions. The AD field has already begun to focus on various aspects of these biological processes and to better understand the cell biology that may be impacted by these genetic signals. Indeed the Cardiff team has begun to use patient-derived inducible pluripotent stem cell (iPSC) systems from individuals with genetic risk profiles as platforms to better understand the biological impact of these signals. It was also suggested that a significant proportion of the genetic variation in disease pathology is yet to be detected and the Cardiff team are using exome chip and next-generation sequencing technology to find both risk genes and AD protective variants. Finally, a novel polygenic scoring system was discussed which was suggested could predict with 90% accuracy which populations have the greatest and least vulnerability to developing AD.

MECHANISMS OF TAU PROPAGATION: RELEVANCE FOR DISEASE MODIFICATION IN AD AND TAUOPATHIES

The first afternoon session was focused on "Tau – The next therapeutic frontier" and was opened with a talk from Dr. Michael O'Neill of Eli Lilly. In his introduction, Dr. O'Neill discussed the different isoforms and phosphorylation states of the tau protein, their contribution to the formation of neurofibrillary tangles and neurite plaques and the role that these are ultimately thought to play in developing AD pathology. It is now well recognized that the extent of the tau pathology in AD correlates much better with cognitive decline and other clinical symptoms than the associated amyloid burden. The contribution that these different tau species also make to other tauopathies, such as progressive supranuclear palsy, frontotemporal dementia and Pick's disease was also highlighted.

The physiological role of tau protein is to bind to and stabilize microtubules. However, when it becomes phosphorylated, tau dissociates from microtubules, causing the axonal structures to weaken, and starts to assemble into the insoluble filaments of hyperphosphorylated protein that then ultimately leads to the formation of neurofibrillary tangles. It was discussed that a growing body of evidence now suggests that tau pathology spreads through the brain in a 'prion-like' manner, starting in the transentorhinal cortex and then moving out to the limbic and ultimately the neocortical regions, resulting eventually in memory loss. This spread of pathology correlates very well with increasing disease severity and performance on cognitive tests and can also be visualized using tau PET imaging (with agents such as T807). The transmission and spreading of pathology can now also be demonstrated in both cellular and animal models of disease. Work by colleagues at Eli Lilly has shown that brain homogenates taken from 5-month-old P301S mice (with high pathological tau burden) and injected into the forebrain of 2-month-old P301S mice where there is no tau pathology, results in a rapid and progressive hippocampal tau pathology in these younger animals (9). It has also been shown that this developing tau pathology spreads to different brain regions, along various network connections.

Dr. O'Neill also presented a cell-based model of tau seeding and propagation in HEK-293 cells (10). Seeding 0N4-Repeat tau (from the sarkosyl-insoluble fraction) from P301S in HEK-293 cells expressing 1N4-Repeat tau led to hyperphosphorylated filamentous aggregates, as visualized by electron microscopy or high content imaging. This model has been used to understand and to study the properties of the seed competent tau and the critical steps that are likely involved in tau aggregation and propagation in vivo. The results indicated that a diverse population of filamentous aggregates are capable of seeding and promoting the spread of tau pathology, with the most potent species being short fibrils (that were present in 40% sucrose fractions). On the basis of data from this cell model, it was shown that total brain lysate and the 40% sucrose fraction, but not a 10% fraction induces tau seeding, spread and propagation in vivo (11).

In the concluding part of his talk, Dr. O'Neill reviewed potential therapeutic strategies to target tau pathogenesis, some of which have been extensively explored. These include microtubule stabilizers (such as epothilone D), tau kinase inhibitors (e.g., glycogen synthase kinase 3β [GSK-3β]), aggregation inhibitors, tau clearance agents (e.g., autophagy inducers) and immunotherapy approaches. The later strategy is based on the hypothesis that aggregated tau in one cell fragments, is released into the extracellular space and is then taken up into neighboring cells, leading to a pattern of trans-synaptic propagation. While in the extracellular space, these tau species can be sequestered by a locally available antibody and removed. Data from Eli Lilly was also presented, showing that a twice-weekly peripheral injection of 15 mg/kg of a monoclonal antibody, PHF-1, into the P301S mouse resulted in reduction in tau pathology at 3 months of age (as measured by AT8 monoclonal antibody [MAb] staining in brain stem and spinal cord) and an associated improvement in motor (as measured on the rotarod) function (12).

In conclusion, Dr. O'Neill indicated that tau drug discovery programs were now established in most major pharmaceutical companies. While very few drug candidates have yet to progress into clinical development, major investments are now going towards establishing cellular and transgenic animal models that recapitulate many aspects of AD in order to aid in the development of these tau-based therapies. Finally, with the development of tau PET tracers, such as flortaucipir (^{18}F-T807, AV-1451) and others, biomarkers of disease progression and a pharmacodynamics marker for future tau therapeutics are becoming much more available.

TAU: AN ATTRACTIVE AND TRACTABLE DRUG TARGET FOR IMMUNOTHERAPY?

In concluding the session, Dr. Marc Mercken from Janssen gave another talk focused on tau. Similar to the previous speaker, Dr. Mercken summarized the contribution of hyperphosphorylated tau and PHF (paired helical filament)-tau to the pathogenic features of AD and explained how these develop in various key brain regions over the different stages of disease. Using new PET imaging agents for both tau and amyloid, it is now possible to clearly visualize the different distribution of these two proteins in the different affected brains of AD patients.

Dr. Mercken proceeded to raise several unresolved issues that exist in the tau field. The first question was whether (monomeric) tau was

hyperphosphorylated in AD. The early AT8 signal in the somatoden-dritic compartment, as observed in the Braak group 1 neurons in the transentorhinal layer, was discussed. This signal is often interpreted as evidence for hyperphosphorylated monomeric tau. A more logi-cal explanation, however, would be that this staining represents the earliest phase of aggregated tau, as post-mortem dephosphoryla-tion and prolonged fixation would have destroyed monomeric AT8 reactivity.

The second question was whether tau is first translocated from axons to neuronal soma in AD, which is then followed by phosphorylation. Data were presented with a high-affinity phospho-tau antibody that showed the selective presence of phospho-tau in the somatoden-dritic compartment of neurons in wild-type mouse tissue, when pro-cessed under controlled fixation and epitope retrieval conditions. It was argued that in normal neurons, sufficient highly phosphorylated tau is present already in the somatodendritic compartment to pro-vide the necessary substrate for continued growth of tau aggregate seeds. The selective incorporation of these most highly phosphory-lated tau monomers would gradually lead to selective accumulation of phospho-tau in AD.

A third question centers around what is the preferred epitope of the widely used antibody tool, AT8. The Fab affinity values of AT8 towards a set of phospho-peptides were determined and the pre-ferred epitope was identified as pS202/pT205/pS208. The inter-action of AT8 with these three phospho-sites was confirmed by crystallography.

Finally, the question around avidity versus conformation and how to see the difference towards aggregate selectivity was posed. As an example of how avidity can lead to strongly increased binding, the K_d values of Fab and IgG of AT8 towards PHF were determined by SPR. The IgG showed ~500 times stronger binding versus the intrin-sic affinity of the Fab. It was pointed out that for all tau antibodies with low intrinsic affinity, a strong avidity effect on PHF binding will be observed which will resemble conformation specific binding. The strong resistance of PHF to post-mortem degradation in AD tissues and the high signal in aggregate versus monomer staining, because of epitope density, makes it very difficult to differentiate conformation-specific binding from avidity effects.

Dr. Mercken went on to highlight the original paper of Braak that first characterized and discussed how tau pathology develops in the human brain over the different decades of life. He then presented an extensive analysis of these data done by Janssen scientists that clearly demonstrates how the presence of increasing amyloid pathol-ogy in AD patients actually accelerates the resulting tau pathology and tau spreading. Based on this and other analysis, a working model named the 'Aggregate Cascade Hypothesis', was proposed to explain the progress of brain pathology. In this cascade, aggregation of Aβ42 initially leads to forms of oligomeric Aβ and deposited amy-loid peptide in plaques. This then produces 'aggregate stress' that interacts with PHF-tau aggregate formation, resulting in a further increased stress that eventually leads to neuronal dysfunction and death, leading ultimately to the clinical signs of dementia. Various potential therapeutic strategies to target tau aggregate stress were proposed, including PHF-tau autophagy-lysosomal clearance, P-tau proteosomal clearance, microtubule stabilization and modulating tau modifications.

In the last part of his talk, Dr. Mercken discussed immunotherapy as an attractive drug target for impacting tau pathology. Reviewing again the transmission and spreading hypothesis, he argued that the presence of tau in the extracellular space after cell death and aggre-gate release provided a good hope for successful antibody-based therapies. Antibody-induced clearance of tau should therefore pro-vide both an efficacious and safe therapy in AD. To achieve this objec-tive within the Janssen program, candidate antibodies were sought that were selective for PHF-tau versus nonphosphorylated tau and had high affinity for the target. Many such PHF-tau-selective MAbs were generated in the program and assessed for functional activ-ity in several mouse models. Of these, one MAb was presented that showed very high affinity binding to PHF-tau with a slow off-rate, and which stained tangles in AD brain versus controls.

This antibody was also tested in the P301S mouse model, starting at 2 months of age, with a twice-weekly injection of 20 mg/kg i.p. At 4 months of age, the PHF-tau load, as measured by AT8 reac-tive aggregates, was significantly reduced. An injection model in P301L transgenic mice, where tau aggregates are injected into the hippocampus and frontal cortex, was also developed in the pro-gram. Aggregation is prominent in the injected hemisphere but also a modest signal can be observed in the noninjected hemisphere. In this model, the MAb showed pronounced effects in reducing the lev-els of PHF-tau.

In conclusion, Dr. Mercken presented a working hypothesis based on antibody-induced microglial clearance of tau seeds, whereby tau released by dying neurons was sequestered by available antibody, blocked from continuing transmission and instead presented for microglial clearance.

INNATE IMMUNITY AS POTENTIAL THERAPEUTIC TARGETS IN AD

Dr. David Brough, from University of Manchester, discussed the cru-cial roles innate immunity and the inflammatory response plays in the pathogenesis of AD. As the major resident immune cells in the brain, microglial cells constantly survey the microenvironment and are activated by and recruited to senile plaques. Subsequently, they can phagocytose β-amyloid and secrete proinflammatory cytokines that influence the surrounding brain tissue. Recently, a wealth of information linking the microglia-specific activation of NLRP3 inflam-masome to AD pathogenesis has emerged. The idea that inflamma-tion contributes to the progression of AD is well documented, and new research points to the NLRP3–inflammasome complex as one of the key regulators of inflammation in AD. The NLRP3 inflammasome forms a molecular platform inside microglia, catalyzing the activa-tion of the protease caspase-1. Caspase-1 is then responsible for con-verting the potent proinflammatory cytokine interleukin-1 (IL-1) from an inactive to an active secreted form. Active caspase-1 is present in the brains of humans with AD, suggesting that NLRP3 may con-tribute to the human condition. Nlrp3$^{-/-}$ or Casp1$^{-/-}$ mice carrying mutations associated with familial AD were largely protected from loss of spatial memory and other sequelae associated with AD, and demonstrated reduced brain caspase-1 and IL-1β activation as well as enhanced β-amyloid clearance (13). Furthermore, NLRP3 inflam-masome deficiency skewed microglial cells to an M2 phenotype and resulted in the decreased deposition of β-amyloid in the APP/PS1

model of AD. These results show an important role for the NLRP3/ caspase-1 axis in the pathogenesis of AD, and suggest that NLRP3 inflammasome inhibition represents a potential novel therapeutic intervention point for the disease.

Current anti-IL-1β drugs such as the biological canakinumab and anakinra do not easily penetrate the brain and there are no molecules known to directly target NLRP3 in clinical use. However, Dr. Brough's group have found that nonsteroidal anti-inflammatory drugs (NSAIDs) of the fenamate class were effective and selective inhibitors of the NLRP3 inflammasome via inhibition of the volume regulated anion channel (VRAC) in macrophages. They characterized several clinically approved and widely used fenamate NSAIDs as NLRP3 inhibitors. Flufenamic and mefenamic acid were efficacious in vivo in a rodent NLRP3-dependent model of inflammation (i.e., subcutaneous urate crystal-induced inflammation) and mefenamic acid also showed efficacy in reversing β-amyloid-induced memory loss (assessed using the rat novel object recognitions test [NOR] of cognition). Moreover, chronic infusion (25 mg/kg/day for 14 days) of mefenamic acid reversed cognitive decline, and attenuated microglial activation and IL-1β expression in the 3xTgAD mouse model. These data suggest that fenamate NSAIDs could be repurposed as NLRP3 inflammasome inhibitors for the treatment of AD. In addition to drug repurposing, Dr. Brough's team are also actively designing and evaluating novel inhibitors of the NLRP3 inflammasome.

Finally, Dr. Brough described some interesting work on identifying lifestyle/dietary factors which may contribute to increased NLRP3-dependent inflammation in AD; in this regard he described their work on dietary Zinc (Zn^{2+}). Zn^{2+} deficiency affects up to 2 billion people worldwide, and is particularly common in aged individuals and aged animals who naturally become Zn^{2+}-deficient possibly due to diet and poor absorption. Zn^{2+} deficiency leads to cognitive impairment in the APP/PS1 AD mouse which can be reversed by addition of supplements. The team has recently reported that Zn^{2+} depletion of macrophages activates the NLRP3 inflammasome in vitro. They are currently investigating the hypothesis that Zn^{2+} deficiency accelerates the aging process by driving NLRP3-dependent inflammation.

AUTOPHAGY UPREGULATION AS A THERAPEUTIC TARGET FOR NEURODEGENERATIVE DISEASES

Autophagy was originally identified as a cytoprotective system that provides emergency backup energy and basic building blocks under starvation condition by digesting self-components. Recent advances in the field have revealed that this system also protects cells against multiple types of stress, as well as invasion by pathogens. Consistent with these findings, autophagy has been redefined as a safeguard system that plays a vital role in human pathology, and this realization has led to exponential progress in autophagy research. In the second session of the afternoon, Dr. Fiona Menzies described the research of the David Rubinsztein lab based at the University of Cambridge.

Intracellular protein aggregation is a feature of many late–onset neurodegenerative diseases including tauopathies, Parkinson's disease and polyglutamine expansion diseases (such as Huntington's disease [HD]). The intracytoplasmic deposition of aggregate-prone proteins in neurons leads to a range of detrimental cellular processes such as mitochondrial dysfunction. Furthermore, many of the mutant proteins associated with these aggregates cause various neurodegenerative diseases via toxic gain-of-function mechanisms. Therefore the factors regulating their clearance are crucial for understanding disease pathogenesis and for developing rational therapeutic strategies.

Macro-autophagy is one of the major intracellular protein degradation pathways. It is initiated by double-membrane structures, which engulf portions of cytoplasm. The resulting autophagosomes ultimately fuse with lysosomes, where their contents are degraded. Dr. Menzie described the basic biology of autophagy before outlining its roles in neurodegeneration, and HD. HD is one of the 10 trinucleotide repeat disorders resulting from expansions of polyglutamine tracts in different proteins. These expansions cause disease by conferring toxic gain-of-function properties onto the mutant proteins. Hence, one strategy that has been considered for HD and related diseases is to find ways of decreasing the levels of the mutant protein, for instance by harnessing the cell's capacity to degrade such aggregate-prone proteins via macro-autophagy.

Dr. Menzies' group have shown that the mTOR inhibitor and autophagy inducer rapamycin reduces the levels of mutant Huntingtin and attenuates its toxicity. In order to induce autophagy long-term, they have been striving to identify safer alternatives to the mTOR inhibitor rapamycin. The focus of their ongoing work has been on the discovery of novel components of the autophagy machinery and new signaling pathways. This has led to the discovery of the inhibition of the type II PI5P kinases as a rational therapeutic strategy for the treatment of neurodegenerative disease. Phosphatidylinositol 3-phosphate (PI(3)P), the product of class III PI3K VPS34, recruits specific autophagic effectors, like WIPI2, during the initial steps of autophagosome biogenesis and thereby regulates canonical autophagy. However, mammalian cells can produce autophagosomes through enigmatic noncanonical VPS34-independent pathways. The Rubinsztein group have shown that PI(5)P can regulate autophagy via PI(3)P effectors and thereby provide mechanistic explanation for forms of noncanonical autophagy (14). PI(5)P synthesis by the phosphatidylinositol 5-kinase PIKfyve is required for autophagosome biogenesis, and it increases levels of PI(5)P, stimulates autophagy and reduces the levels of autophagic substrates. Inactivation of VPS34 impairs recruitment of WIPI2 and DFCP1 to autophagic precursors, reduces ATG5-ATG12 conjugation and compromises autophagosome formation. However, these phenotypes can be rescued by PI(5)P in VPS34-inactivated cells. These findings provide a mechanistic framework for alternative VPS34-independent autophagy-initiating pathways, like glucose starvation, and may unravel a cytoplasmic function for PI(5)P, which previously has been linked predominantly to nuclear roles.

DISCLOSURES

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