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Beyond Indigestion: Emerging Roles for Lysosome-Based Signaling in Human Disease

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Abstract

Lysosomes are becoming increasingly recognized as a hub that integrates diverse signals in order to control multiple aspects of cell physiology. This is illustrated by the discovery of a growing number of lysosome-localized proteins that respond to changes in growth factor and nutrient availability to regulate mTORC1 signaling as well as the identification of MiT/TFE transcription factors (MITF, TFEB and TFE3) as proteins that shuttle between lysosomes and the nucleus to elicit a transcriptional response to ongoing changes in lysosome status. These findings have been paralleled by advances in human genetics that connect mutations in genes involved in lysosomal signaling to a broad range of human illnesses ranging from cancer to neurological disease. This review summarizes these new discoveries at the interface between lysosome cell biology and human disease.

Introduction

Lysosomes degrade and recycle a broad range of macromolecules. This fulfills a housekeeping function and serves as an important source of nutrients. To meet these demands, the lysosomal lumen possesses an array of hydrolytic enzymes that digest complex biological macromolecules to regenerate the basic building blocks of cells. The diverse collection of proteins that are required to support these degradative functions of lysosomes is illustrated by the >50 distinct human diseases (collectively known as lysosomal storage disorders) that arise due to mutations that affect the ability of lysosomes to degrade and recycle their many substrates (reviewed in: [1–3]). While the digestive role of lysosomes is of unquestionable importance, the emergence of signaling functions for lysosomes demonstrates an even broader influence of lysosomes on cellular physiology that has an impact on a wide range of human diseases that extends beyond the lysosome storage diseases that have traditionally been associated with the dysfunction of this organelle. This review thus focuses on emerging aspects of lysosome-localized regulation of mTORC1

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signaling and the lysosome-to-nucleus signaling mediated by MiT/TFE transcription factors. The contributions of such signaling to diverse physiological processes is further highlighted by the increasing number of human illnesses that arise due to mutations in the genes encoding lysosome-localized signaling proteins.

Lysosomes coordinate mTORC1 signaling

The mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway plays a broad role in the regulation of cell metabolism and growth [5–7]. mTORC1 activity is influenced by multiple inputs including growth factor signaling and the intracellular abundance of: ATP, oxygen, glucose and amino acids to control the balance between anabolic processes such as protein translation and catabolic cellular processes such as autophagy and lysosome biogenesis. While the signaling functions of mTORC1 have long been appreciated, lysosomes have more recently been identified as the major intracellular site where growth factor and nutrient signals converge to activate mTORC1 (Figure 1). The following sections will: 1) Summarize the lysosome-localized actions of Rheb and Rag GTPases that act in parallel to ensure that maximal mTORC1 activation is coordinated with growth factor and nutrient availability (Figure 1); and 2) Describe how mutations in such lysosome-localized signaling proteins contribute to human disease (Table 1). While this review focuses specifically on links between lysosomal signaling mechanisms and human disease, many aspects of the lysosomal nutrient sensing pathways and their links to the Tor kinase are evolutionarily conserved and investigation of these processes in model organisms has greatly contributed to our understanding of such pathways [4].

Regulation of mTORC1 activation by Rheb

The kinase activity of mTORC1 is stimulated by direct interactions of mTOR with the GTP-bound form of the Rheb GTPase [8], a protein that resides on the cytoplasmic surface of lysosomes [9]. This interaction is inhibited by the Rheb-GTPase activating protein (GAP) activity of the tuberous sclerosis protein complex (TSC) that is formed by the TSC1, TSC2 and TBC1D7 proteins [10]. The actual GAP activity of this trimeric complex is mediated by TSC2 while both TSC1 and TBC1D7 are critical for TSC complex stability [10,11]. Growth factors, such as insulin, stimulate mTORC1 activity via the receptor tyrosine kinase (RTK)-PI3K-AKT signaling pathway that culminates in the AKT-mediated phosphorylation of TSC2 [12–14]. This phosphorylation disrupts TSC-Rheb interactions and causes TSC to dissociate from the surface of lysosomes [15]. Conversely, under conditions of energy stress, the AMP-activated protein kinase (AMPK) suppresses mTORC1 activity by phosphorylating a different site on TSC2 that results in enhanced GAP activity towards Rheb [16]. Thus, regulating the lysosomal localization and Rheb-GAP activity of a complex comprising TSC1-TSC2-TBC1D7 is of key importance for controlling mTORC1 signaling.

Rheb hyperactivation results in diseases of uncontrolled cell growth

Inactivating mutations in TSC1 and TSC2 (also known as hamartin and tuberlin respectively) cause both tuberous sclerosis complex and lymphangioleiomyomatosis (LAM). These diseases are characterized by hyperactive mTORC1 signaling and the aberrant growth of cells in multiple tissues [17,18]. The neurological consequences of tuberous sclerosis

complex can be severe and include seizures in the majority of patients along with varying degrees of cognitive impairment and strong autism predisposition [17,19]. The causative role of excessive mTOR activity in epilepsy is further supported by the recent identification of somatic activating mTOR mutations in the brains of patients suffering from focal cortical dysplasia type II, a form of epilepsy that is often resistant to anticonvulsant medications [20]. Human genetic studies within the last 2 years have additionally identified rare loss-of-function mutations in TBC1D7 that do not cause the full range of tuberous sclerosis complex symptoms but nonetheless result in excessive mTORC1 activity and the development of megalencephaly combined with intellectual disability [21,22]. There is also a potential link between TBC1D7 and migraine susceptibility [23]. Additional studies are required to elucidate the mechanisms that lead to the multiple distinct human diseases that arise from TSC1, TSC2 and TBC1D7 mutations and their potential relevance to signaling from the lysosome. In addition to the direct consequences of mutations in the genes encoding TSC proteins, this lysosome-localized protein complex is also a downstream target of numerous oncogenes (including: RTKS, PI3K, AKT, Ras, Raf) and tumor suppressors (including: PTEN, NF1, LKB1) whose mutations collectively contribute to the elevation of mTORC1 signaling in a large fraction of human cancers [5,24]. mTOR as well as its upstream regulators thus represent a potentially important therapeutic target in a wide range of diseases that are characterized by hyperactive mTORC1 signaling. As proof of principle, cancer patients have recently been identified that exhibit exceptional responses to mTORC1 inhibitors that have been attributed to the major roles played by specific mTORC1 pathway mutations in driving the growth of their tumors [25–27].

Acute regulation of the Rag GTPases controls the recruitment of mTORC1 to lysosomes

Rag GTPases assemble as heterodimers made up of either RagA or RagB bound to RagC or RagD that localize to the cytoplasmic surface of lysosomes. Recruitment of mTORC1 to the surface of lysosomes through interactions with Rag heterodimers positively regulates mTORC1 activity by bringing mTOR into proximity with Rheb [28–30]. As Rag-mediated recruitment of mTORC1 to lysosomes is tightly regulated by intracellular amino acid availability (Figure 1), considerable interest is currently focused on the mechanisms whereby amino acid availability regulates the Rag GTPases.

The nucleotide status of each individual Rag protein within a Rag heterodimer influences mTORC1 recruitment to lysosomes with the most active form represented by the combination of RagA/B^{GTP}-RagC/D^{GDP}[29]. A membrane anchored complex, the Regulator, made up of Late Endosomal/Lysosomal Adaptor, MAPK And MTOR Activator 1 (LAMTOR1/p18), LAMTOR2/p14, LAMTOR3/MP1, LAMTOR4/C7orf59 and LAMTOR5/HBXIP tethers Rag heterodimers to the surface of lysosomes and also acts as a guanine nucleotide exchange factor (GEF) for Rag A/B [28,31]. This function of the Regulator is intimately dependent on lysosome status via physical interactions with the V-ATPase [31]. Both the V-ATPase as well as the proton gradient that it generates are required for the amino acid stimulated GEF activity of Regulator towards RagA/B [31]. In addition to their role in the mTORC1 pathway, several Regulator components (LAMTOR1/p18,

LAMTOR2/p14, LAMTOR3/MP1) were also separately identified as a scaffold for the recruitment of mitogen activated protein kinase (MAPK) to the surface of late endosomes-lysosomes [32,33]. It remains to be determined whether this represents a mechanism for cross-talk between MAPK and mTORC1 signaling pathways or more general roles for lysosomes as either regulators or targets of MAPK signaling.

The active, GTP-bound, state of RagA/B is opposed by the GAP activity of a separate lysosome-localized complex, GATOR1, made up of Nprl2, Nprl3 and DEPDC5 [34]. This activity of GATOR1 is in turn negatively regulated by a poorly understood pentameric complex (comprised of Sec13, Seh11, Mios, Wdr24 and Wdr59) known as GATOR2 [34,35,36]. While the specific mechanisms of GATOR2 function remain uncertain, this complex is predicted to have structural similarity to other protein complexes that form coats at membrane interfaces such as the COPII coat and nuclear pores [37]. Indeed, in addition to functioning in GATOR2, Sec13 is also part of both COPII coats and nuclear pores while Seh11 has shared roles in both GATOR2 and nuclear pore complexes. GATOR2 is regulated by interactions with the sestrins that are controlled by amino acid availability [35,36]. As sestrins also independently inhibit mTORC1 in response to various cellular stresses via an AMPK-TSC2 dependent mechanism [38], there is the potential for cross talk between these pathways.

Additional Rag regulation occurs via a dimeric complex made up of folliculin (FLCN) and either of the homologous FLCN-interacting proteins 1 or 2 (FNIP1/2) that interacts directly with the GTPase domain of RagA/B in a nutrient regulated manner [39]. Surprisingly, given an FLCN crystal structure [40] and subsequent bioinformatics analysis [41] that predicted that the major folded domain of both FLCN and FNIP1/2 most closely resembles the DENN family of Rab GEFs, biochemical analysis has detected GAP activity of the FLCN-FNIP complex towards RagC/D [42]. As FNIP1/2 also interacts strongly with AMPK [43,44] and FLCN depleted cells exhibit elevated AMPK signaling [45], the FLCN-FNIP1/2 complex may further serve to mediate cross talk between AMPK and mTORC1 signaling pathways.

The precise mechanisms whereby specific intracellular amino acids (leucine, glutamine and arginine in particular) are sensed remain unclear [46] but amino acid responsiveness of mTORC1 signaling is at least in part dependent on SLC38A9, an arginine and/or glutamine transporter of the lysosomal membrane that co-purifies with the Ragulator and the Rags [47,48]. Physical interactions between the lysosomal V-ATPase with multiple Ragulator subunits also contribute to amino acid sensing mechanisms [31,49]. While Rags play a prominent role in communicating amino acid availability to mTORC1, RagA/B knockout cells were recently shown to retain a residual responsiveness of mTORC1 to glutamine that is dependent on the Arf1 GTPase [50]. Thus, multiple mechanisms appear to function in parallel to support the amino acid dependence of mTORC1 signaling.

Rag GTPase dysregulation and human disease

Defects in the ability of lysosomes to regulate Rag GTPases in response to changes in intracellular amino acid availability have recently been linked to several human diseases. Loss-of-function FLCN mutations cause Birt-Hogg-Dubé syndrome, a disease characterized

by the growth of hair follicle tumors (fibrofolliculomas), lung cysts, spontaneous pneumothorax (collapsed lung) and a high incidence of renal carcinoma [51]. Exome sequencing also recently identified an FLCN mutation in combination with a TSC2 mutation in anaplastic thyroid cancer with dysregulated mTORC1 signaling and robust responsiveness to treatment with mTORC1 inhibitors [27]. The extent to which such sporadic FLCN mutations contribute to cancers beyond Birt-Hogg-Dubé syndrome warrants further investigation.

The human Rag GTPase pathway is hyper-activated by loss-of-function mutations in DEPDC5 and Nprl2 (components of the GATOR1 RagA/B GAP). DEPDC5 mutations cause multiple neurological defects including: familial focal epilepsy, abnormal neuron growth and brain malformations such as hemimegalencephaly [52–56]. Loss of DEPDC5 thus shares mechanistic and neurologic similarities with TSC mutations that also result in excessive mTORC1 activity and seizure susceptibility. Meanwhile, NPRL2 has a tumor suppressor function and deletions of this gene have been observed in multiple cancers including lung cancer and glioblastoma as well as in some cancer derived cell lines [34,57]. As NPRL2 and DEPDC5 are thought to function as part of the same RagA/B GAP complex, it is not yet clear how their mutations result in different diseases.

Point mutations in the 3' untranslated region of the LAMTOR2/p14 (Regulator component) gene that result in reduced protein expression were identified in members an isolated family suffering from a constellation of symptoms including: immune deficiency, hypopigmentation and short stature [58]. Characterization of cells from such patients revealed defects in the intracellular positioning of late endosomes/lysosomes [58] and subsequently the critical contributions of LAMTOR2/p14 to the recruitment of Rag GTPases to the cytoplasmic surface of lysosomes [28].

Hypomorphic mutations in VMA21, a gene encoding a chaperone that promotes V-ATPase assembly within the endoplasmic reticulum result in X-linked myopathy with excessive autophagy (XMEA) [59]. Reduced V-ATPase levels result in impaired lysosome acidification in XMEA patients. Diminished mTORC1 signaling in XMEA has also been observed and attributed to reduced generation of amino acids via lysosome-mediated proteolysis [59] but could potentially also reflect impaired transduction of amino acid-dependent signals to mTORC1.

Regulation of lysosome homeostasis by the lysosome-to-nucleus shuttling of MiT/TFE transcription factors

A major advance in the field of lysosome cell biology was the discovery that transcription factor EB (TFEB, a basic-helix-loop-helix-leucine zipper transcription factor) can coordinate the expression of numerous genes encoding lysosome and autophagosome proteins by binding to an E-box-like response element (CLEAR element) found within their promoters [60,61]. This ability of TFEB to regulate lysosome function has been investigated as a potential therapy for lysosomal storage diseases [62–64] as well as for the clearance of protein aggregates in neurodegenerative diseases [65–67] and has shown promise in both cell culture and animal models.

In addition to investigating the therapeutic potential of TFEB-mediated enhancement of the lysosomal pathway, considerable progress has been made in understanding the mechanisms whereby cells normally regulate this transcription factor. Under basal conditions of intact lysosome function and adequate nutrient availability, TFEB is largely maintained in an “off” state. This is achieved by the dynamic recruitment of TFEB to the cytoplasmic surface of lysosomes via Rag GTPase interactions whereupon TFEB can be phosphorylated on serine 211 in an mTORC1-dependent manner and subsequently released from lysosomes and sequestered in the cytoplasm by phosphorylation-dependent interactions with 14-3-3 proteins [68–71]. In response to either impaired lysosome function and/or the depletion of intracellular amino acids, this cytoplasmic sequestration of TFEB is relieved and TFEB translocates to the nucleus to elicit an adaptive response [68–70]. More recently, calcineurin was identified as a phosphatase that can respond to lysosomal calcium signals to promote the nuclear localization of TFEB via dephosphorylation of serine 211 [72]. While such signaling plays a powerful role in controlling the nucleus versus cytoplasmic localization of TFEB via regulation of its phosphorylation on serine 211 [68,69], other TFEB phosphorylation sites have been characterized [61,73,74] and large scale phospho-proteomic studies (www.phosphosite.org) have identified numerous additional TFEB phosphorylation sites whose functions and relationship to lysosome status remain to be determined.

TFEB shares extensive sequence homology with transcription factor E3 (TFE3) and the microphthalmia transcription factor (MITF). Both MITF and TFE3 dimerize with TFEB (as well as each other), are regulated by lysosome status in a manner that parallels TFEB [69] and also regulate the expression of genes encoding lysosomal proteins [64,75]. MITF is phosphorylated by both MAPK [76] and Wnt [75] signaling pathways on sites that are conserved in both TFEB and TFE3, however, it remains to be determined how such phosphorylation is either spatially or functionally connected to lysosomes. In this light, the MAPK recruitment to late endosomes/lysosomes by regulator subunits [32,33] is particularly intriguing and warrants further investigation.

MiT/TFE Transcription Factors are oncogenes

Chromosomal translocations involving the TFEB, TFE3 and MITF genes cause renal carcinoma and other cancers [77–79]. While these chromosomal translocations frequently result in the expression of a fusion protein that could theoretically cause cancer through novel gain-of-function mechanisms, specific chromosomal translocations have been identified that place TFEB expression under the control of a much stronger promoter (~60 fold up-regulation) without changing the TFEB coding sequence [79,80]. Thus, elevated expression of the wildtype TFEB protein is sufficient for the oncogenic mechanism. More subtly, point mutations that impair MITF sumoylation enhance risk for both renal carcinoma and melanoma [81]. There is also a rich literature concerning the many roles played by MITF as a master regulator of the melanocyte lineage [82]. It remains to be determined how the cancer causing effects of excessive levels of MiT/TFE transcription factors relates to their roles as regulators of lysosome function. These transcription factors also have multiple targets with cancer relevance whose regulation by lysosome status remains unknown including: E-cadherin [83], a component of adherens junctions; CDK2, a kinase that regulates the cell cycle [84]; PGC1 α , a transcriptional co-activator that regulates metabolism

and mitochondrial function [85]; and Met, a receptor tyrosine kinase whose activating mutations are also common in renal carcinoma [77,86].

Possible contributions of MiT/TFE transcription factors to Birt-Hogg-Dubé syndrome

As mTORC1 activity is elevated in the majority of cancers [5,24], it is counterintuitive that FLCN is both a tumor suppressor in Birt-Hogg-Dubé syndrome as well as a positive regulator of mTORC1 activity via direct Rag interactions [39,42]. However, as over-expression of MiT/TFE transcription factors causes cancer independent of mutations to canonical upstream components of the mTORC1 signaling pathway [77,78], the increased nuclear levels of MiT/TFE transcription factors may be a more important contributor to tumor growth in Birt-Hogg-Dubé syndrome than the negative effects of FLCN depletion on mTORC1 signaling [39,42]. Indeed, enhanced nuclear localization of MiT/TFE proteins has been widely observed in FLCN-depleted cells [39,87,88], while effects on the mTORC1 pathway vary dramatically depending on cell culture versus in vivo tumor contexts and may reflect long term compensatory changes required for neoplastic growth [89–91]. The exquisite sensitivity of MiT/TFE nuclear abundance to FLCN loss may arise from the double dependence of MiT/TFE proteins on the Rag GTPases for their negative regulation (Figure 2). Inhibition of the nuclear localization of MiT/TFE proteins requires Rags for both their own recruitment to lysosomes (a prerequisite for their mTORC1-dependent phosphorylation) as well as for the lysosomal recruitment and activation of mTORC1 whose kinase activity is required for MiT/TFE phosphorylation and cytoplasmic sequestration [69,71]. In contrast, other key mTORC1 targets such as ribosomal protein S6 kinase and 4E-BP1 are directly recruited to mTORC1 via their direct binding to the raptor subunit [92,93] and thus do not exhibit a synergistic dependence on the Rags for both their mTORC1 recruitment and phosphorylation. It has recently been reported that cells from RagA/B conditional knockout mice exhibit excessive cell growth/proliferation [94,95]. Based on the model outlined above, excess MiT/TFE activity represents a candidate for explaining these unexpected phenotypes.

Conclusions

Lysosomes serve as a platform for a growing collection of proteins that integrate multiple signals that control the balance between anabolic and catabolic cellular processes. mTORC1 activity is the major output of growth factor signaling and nutrient sensing mechanisms that converge at the lysosome that is in turn influenced at multiple levels by energy availability via AMPK. The MiT/TFE transcription factors are regulated by lysosome status through a dual mechanism that involves both a direct interaction with the lysosome-localized Rag GTPases as well as their mTORC1-dependent phosphorylation. Looking towards the future, the currently high pace of discovery of lysosome-localized proteins required for mTORC1 signaling will need to be complimented with greater insight into the mechanistic basis for their actions. Such advances will provide a strong foundation for developing therapeutic tools to address the expanding range of human diseases that arise from the dysfunction of lysosome-based signaling pathways. Coming full circle back to the longstanding problem of

lysosome storage disorders and their pathogenetic mechanisms, while it intuitively makes sense that the accumulation of undigested material within the lysosomal lumen can elicit adverse effects on cell health, the mechanisms that link storage of specific molecules with distinct disease pathologies or the vulnerability of different cell types to specific degradation defects is not well understood. A better understanding of the impact of lysosomal storage on lysosome-based signaling mechanisms might help to address these issues and could also offer therapeutic opportunities.

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Highlights

- Lysosomes integrate multiple signals required for mTORC1 signaling.
- Lysosomal signals regulate gene expression through MiT-TFE transcription factors.
- Multiple diseases arise from mutations in lysosomal signaling protein genes.

Figure 1. Multiple signals converge on the surface of lysosomes to regulate mTORC1 activity
A) Confocal imaging reveals the dramatic recruitment of mTOR (green) to lysosomes (LAMP1, red) following the acute re-feeding (20 minutes) of amino acid starved (2 hours) HeLa cells (scale bar = 10 μ m, Image provided by Agnes Rocznik-Ferguson). **B)** This schematic diagram depicts key lysosome localized proteins that coordinate the regulation of mTORC1 activity in response to ongoing changes in nutrient, energy and growth factor availability. Proteins or protein complexes that positively and negatively contribute to mTORC1 activity are shaded green and red respectively. Arrows indicate the actions of

specific proteins/protein complexes on their immediate downstream targets (pointed arrows = stimulation of target; blunt arrows = inhibition of target). “AA” indicates sites that have been identified as targets of regulation by amino acid availability.

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Figure 2. MiT/TFE transcription factors are regulated by Rag GTPases via both direct and mTORC1-dependent mechanisms

This schematic diagram summarizes the synergistic regulation of MiT/TFE transcription factors by Rag GTPases through both the direct Rag-MiT/TFE interactions that recruit these transcription factors to lysosomes as well as the role played by the Rags in the activation of mTORC1 and subsequent MiT/TFE phosphorylation. In contrast, mTORC1 targets involved in the regulation of translation and autophagy do not exhibit direct Rag interactions. Green arrows = positive regulation; Red arrows = inhibition.

Table 1

Summary of genes encoding lysosomal signaling proteins with established roles in human disease.

Gene	Disease	Inheritance/Genetic Mechanism	Molecular Function
TSC1	Tuberous Sclerosis Lymphangioleiomyomatosis	Autosomal	Component of TSC Rheb GAP complex
TSC2	Tuberous Sclerosis Lymphangioleiomyomatosis Cancer	Autosomal Dominant	Rheb-GAP
TBC1D7	Megalencephaly Intellectual Disability Migraine susceptibility?	Autosomal Recessive	Component of TSC Rheb GAP complex
DEPDC5	Familial Focal Epilepsy Hemimegalencephaly Cancer	Autosomal Dominant – Second hit mutation in affected cells Somatic Mutations	Component of GATOR1 – RagA/B GAP complex
Npr12	Cancer	Mutations in various tumors and cancer-derived cell lines	Component of GATOR1 – RagA/B GAP complex
LAMTOR2/p14	Primary Immunodeficiency with short stature and hypopigmentation	Autosomal recessive – Reduced expression.	Component of Ragulator–RagA/B GEF
VMA21	X-Linked Myopathy with Excessive Autophagy	X-linked, hypomorphic mutations	Chaperone for V-ATPase assembly
FLCN	Birt-Hogg-Dubé Syndrome	Autosomal dominant (loss-of-heterozygosity in affected tissue)	RagC GAP
TFEB	Renal Carcinoma	Chromosomal translocation (somatic)	Transcription factor
TFE3	Renal Carcinoma; Alveolar soft part sarcoma	Chromosomal translocation (somatic)	Transcription factor
MITF	Melanoma; Renal Carcinoma	Chromosomal translocation (somatic); also point mutation	Transcription factor