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Cellular recycling gone wrong: The role of dysregulated autophagy and hyperactive mTORC1 in the pathogenesis of sarcoidosis

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Abstract. *Background and aims:* Autophagy is a highly regulated, complex intracellular recycling process that is vital to maintaining cellular homeostasis in response to diverse conditions and stressors. Despite the presence of robust regulatory pathways, the intricate and multi-step nature of autophagy creates opportunity for dysregulation. Errors in autophagy have been implicated in the development of a broad range of clinical pathologies including granulomatous disease. Specifically, activation of the mTORC1 pathway has been identified as a key negative regulator of autophagic flux, prompting the study of dysregulated mTORC1 signaling in the pathogenesis of sarcoidosis. *Our review:* We conducted a thorough search of the extant literature to identify the regulatory pathways of autophagy, and more specifically the implication of upregulated mTORC1 pathways in the pathogenesis of sarcoidosis. We review data showing spontaneous granuloma formation in animal models with upregulate mTORC1 signaling, human genetic studies showing mutation in autophagy genes in sarcoidosis patients, and clinical data showing that targeting autophagy regulatory molecules like mTORC1 may provide new therapeutic approaches for sarcoidosis. *Conclusions:* Given the incomplete understanding of sarcoidosis pathogenesis and the toxicities of current treatments, a more complete understanding of sarcoidosis pathogenesis is crucial for the development of more effective and safer therapies. In this review, we propose a strong molecular pathway driving sarcoidosis pathogenesis at which autophagy is at the center. A more complete understanding of autophagy and its regulatory molecules, like mTORC1, may provide a window into new therapeutic approaches for sarcoidosis.

Key words: autophagy, granulomatous disease, sarcoidosis, mTOR, treatment

Background

Sarcoidosis is the most common diffuse parenchymal lung disease of young adults, characterized by granuloma formation in various compartments of the lung. Granulomatous inflammation can also occur in other organs such as the skin, eye, and liver, and less commonly the heart and brain. In contrast to most other lung diseases, clinical reviews and practice guidelines of sarcoidosis are notable for the lack of new therapies, linked to the incomplete understanding of disease pathogenesis (1,2). Recent progress into identifying the molecular mechanisms and signaling pathways that dictate granuloma formation and progression has the potential to revolutionize therapy for sarcoidosis. In this review we will summarize the growing experimental and clinical evidence supporting

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a key role for defective autophagy in sarcoidosis and discuss the therapeutic potential of drugs capable of reversing these defects with the goal ultimately to improve the care of patients who suffer from sarcoidosis.

Autophagy

Autophagy is a lysosome-dependent evolutionarily conserved process that breaks down and recycles dysfunctional biomolecules, organelles, and intracellular pathogens to retain cellular homeostasis. In autophagy, phagophores engulf and fuse cytoplasmic elements targeted for recycling. On a molecular level, two ubiquitin-like conjugation systems are responsible for the initiation and maturation of the phagophore. The first is the conjugation of Atg5-Atg12, which goes on to form a large complex with Atg16L1 and is essential in phagophore elongation but dissociates after formation of the autophagosome. The second system involves LC3, which following molecular processing via lipidation, inserts and remains in the expanding phagophore membrane where it mediates maturation of the developing autophagosome (3,4). Beyond these well-defined integral systems for the autophagy process, the greater autophagic machinery requires a vast collection of functional molecular interactions under the control of multiple signaling pathways (Figure 1a). A proteomic analysis performed by Behrends et. al., defined the autophagic network of interactions under basal autophagic conditions to include 751 interactions among 409 candidate proteins(5). While many of these interactions and regulatory mechanisms have been defined, a significant amount remains poorly understood or unknown.

Although several signaling pathways control autophagy (4), the classic regulatory mechanism of autophagy is anchored by the mammalian target of rapamycin (mTOR) kinase. The mTOR pathway, responding to signals of cellular plenty such as ATP, amino acids, insulin, and growth factors, promotes cell growth, proliferation, and protein synthesis, exerting a tonic inhibitory effect on phagophore formation via Ulk1 (6). AMPK and mTOR coordinate the regulation of Ulk1 and mammalian autophagy initiation, (Figure 1b) and on the master transcription factor for autophagy genes, TFEB1 (7). In states of nutrient depletion, cellular stress, or lysosomal dysfunction (8) mTOR is inhibited, unleashing autophagy initiation and completion (Figure 1c).

Autophagy and sarcoidosis pathogenesis

There is increasing evidence that implicates autophagy in either the pathogenesis of or the protection from a multitude of diseases including cancers or their response to chemotherapy, neurodegenerative diseases characterized by accumulation of damaged proteins, infectious diseases characterized by defective clearance of pathogens, and auto-immune diseases (9). These observations have stimulated a particular interest in understanding the role of autophagy in conditions characterized by granulomatous inflammation as a mechanism to contain mycobacteria, fungi, or foreign material. Sarcoidosis is an immune disease characterized by granuloma formation in posited to occur in response to environmental and infectious triggers, as suggested by epidemiological studies and by murine models of granulomatous disease (10–15). Defects in autophagy would support a model in which both of these disparate causes could incite granuloma formation in susceptible individuals. We review herein recent studies that demonstrate an important role of autophagy and mTOR signaling in the initiation and maintenance of granulomas, supporting of a key role of autophagy in sarcoidosis.

A well-known driver of granulomatous inflammation, *Mycobacterium tuberculosis*, has been found to inhibit phagosome maturation in macrophages, an essential step in autophagy completion and clearance of bacteria and cellular debris (16). Further, stimulation of autophagy induction using either nutrient depletion or with mTOR inhibition by rapamycin was sufficient to overcome mycobacterial (BCG) inhibition of phagolysosome maturation and reduced mycobacterial viability in infected macrophage cell lines (17). Improved autophagy achieved by inhibition of sphingomyelinases and ceramide was also shown to aid BCG clearance in macrophages (18). Although not directly tested in granulomatous inflammation, it is important to note a key role of the lysosomal acid sphingomyelinase and ceramidase in maintaining mTOR activity, where a marked induction in autophagy can be achieved by sphingomyelinase knockdown or inhibition (such as by tricyclic antidepressant imipramine) (8). Additional supporting evidence was the finding of highly upregulated mTORC1 dependent signal transduction in human granuloma formation in a model wherein mononuclear cells were exposed to M. tuberculosis antigens (19).

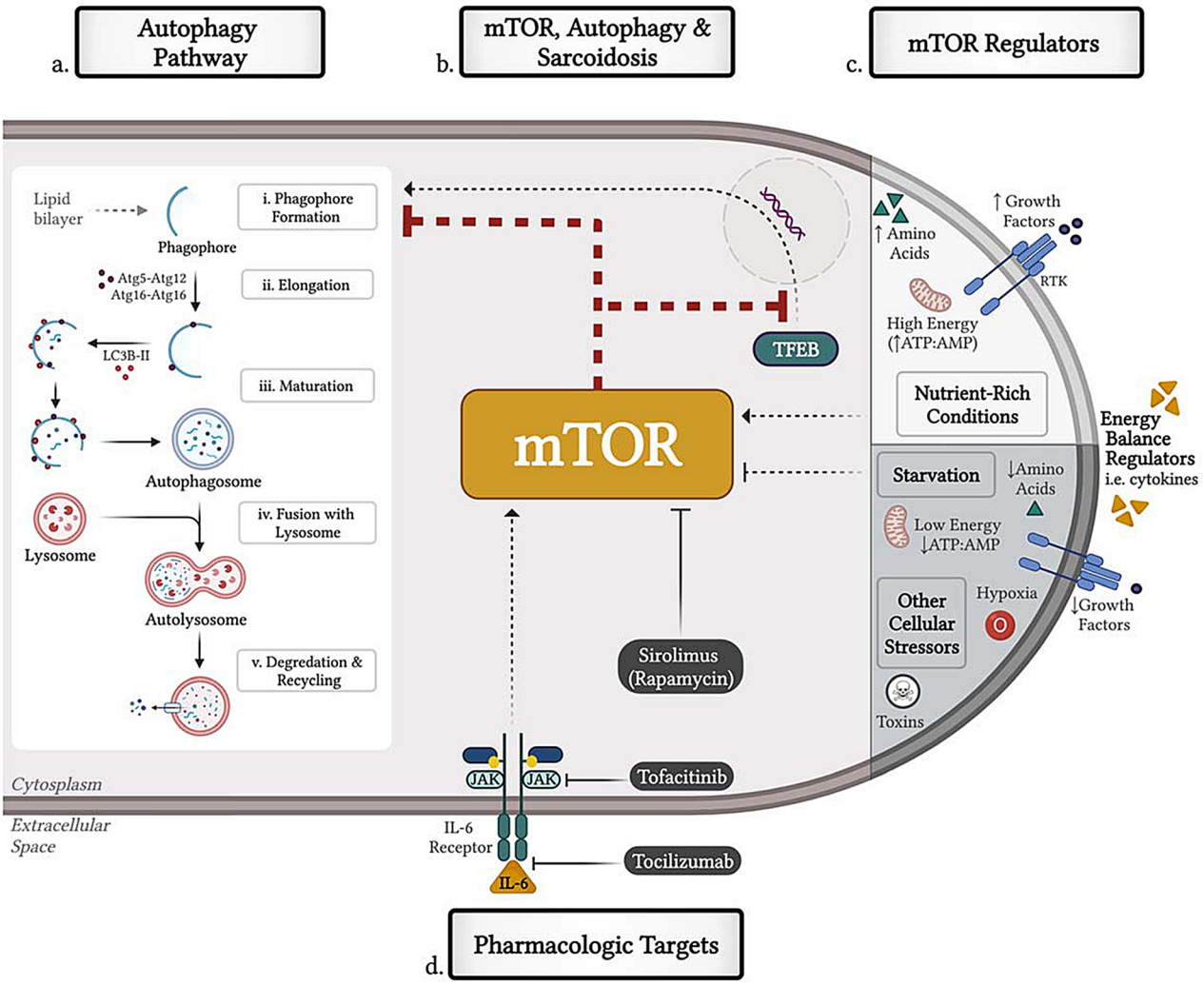

Fig. 1. Autophagy, mTOR, and Sarcoidosis. a) Autophagy is a highly regulated, complex multi-step (i-v) self-degradative process critical for cellular homeostasis. b) The mTOR pathway is a key regulatory mechanism for autophagy via inhibition of several stages of autophagy. Insufficient or dysfunctional autophagy, induced by excessive activation of the mTOR pathway, may be a key driver of the granulomatous inflammation in sarcoidosis. c) The mTOR pathway is activated by markers of cellular plenty and inactivated in states of nutrient-depletion and stress. d) Pharmacologic agents that increase autophagy activity through inhibition of mTOR may provide new therapeutic options for sarcoidosis. These include the direct mTOR inhibitor sirolimus and indirect mTOR inhibitors, such as Tocilizumab (an IL-6 receptor inhibitor) and Tofacitinib (a JAK inhibitor). (*Figure 1 created with biorender.com).*

In a mouse model for constitutive mTORC1 activation in myeloid cells by targeting the upstream inhibitor TSC2 (20), Linke et. al. found that the myeloid deletion of TSC2 in 3-month-old mice induced the spontaneous formation of non-caseating granulomatous aggregates predominantly in the lungs and liver, as well as in lymph nodes, with histopathologic similarity to those found in sarcoidosis. These findings were corroborated by enhanced cell proliferation, hypertrophy, and granulomatous aggregation in TSC2-deficient bone marrow-derived macrophages (BMDM). The granulomatous phenotype was fully reversable both in vitro and in vivo using the mTOR inhibitor rapamycin and the potent mTORC1 inhibitor everolimus, respectively. To clarify the mechanism driving this phenotype, transcriptome analysis was done with TSC2-deficient BMDMs and found 401 genes up-regulated greater than 2-fold, and 426 down-regulated. When compared with BDMDM WT controls, significant changes were found in genes regulating glycolysis, oxidative phosphorylation, cell-cycle proliferation, and inhibition of

apoptosis, favoring proliferation and survival. These findings in mice were corroborated by evidence of mTORC1 activation in a third of 27 mediastinal lymph node biopsies from sarcoidosis patients (20). In a re-analysis of tissue obtained from transbronchial biopsies from 15 patients with sarcoidosis (21), GSEA found the mTORC1 pathway significantly enriched in tissue from patients with progressive disease compared with tissue from patients with self-limited disease.

Other research groups reported highly active mTORC1 signaling measured by downstream effectors S6K1 and 4EBP1 in all 58 patients with biopsy proven sarcoidosis studied, although no significant correlation between mTOR activation and disease severity or need for treatment was detected (22). Similar findings of mTOR gene enrichment was found in a genome wide analysis of familial cases of sarcoidosis (23). Taken together, these data provide strong support that incomplete or inappropriate inhibition of autophagy by a hyperactive mTOR signaling pathway may play an important role in the pathogenesis of sarcoidosis.

Autophagy and sarcoidosis epidemiology

Epidemiologic study has contributed greatly to our understanding of the distribution, risk factors, and outcomes of sarcoidosis. Epidemiologic patterns and variations in the quality of cellular autophagy have also emerged, yielding opportunities to corroborate the link between sarcoidosis and dysfunctional autophagy. It is well known that in the United States, sarcoidosis disproportionately affects African Americans (AA), particularly AA women. Population based studies have shown that AA individuals have a three to fourfold higher risk of disease compared to Caucasians and are more likely to experience progressive or extra-thoracic disease necessitating corticosteroid treatment (24). Overall mortality rates have also been shown to be disproportionately higher in women compared to men, and in AA compared to Caucasians. Interestingly, studies of women with insulin resistance found that AA women exhibited decreased levels of LC3, a validated marker of steady state autophagic activity, suggesting diminished basal autophagy in AA women, although this was accompanied by increased levels of Beclin-1, a key autophagy regulator (25). A study of keratinocytes also suggesting diminished autophagic activity of melanosomes in AA (26) supporting the notion of genetic modifiers of basal autophagy that may parallel the sarcoidosis predilection in AA individuals.

Further evidence of genetic predisposition in sarcoidosis is provided by the occasional familial clustering of disease. A study of 23,800 patients with sarcoidosis in Sweden found that having at least one first-degree relative with sarcoidosis increased 3.7-fold the risk of being diagnosed with sarcoidosis (95% CI 3.4–4.1) (27). Remarkably, whole exome sequencing of 14 individuals with a positive family history of sarcoidosis with an autosomal dominant inheritance pattern compared to controls found 227 disease susceptibility variants in 192 genes, most enriched in autophagy, with the highest-ranking pathways being mTOR and phenylethylamine degradation (23).

Evidence demonstrates sex differences in autophagy (28,29). Several genes on the X chromosome participate in or affect autophagy activity and sex hormone receptors influence the transcriptional regulation of autophagy genes (30). While the molecular mechanisms by which sex influence autophagy remain incompletely defined, such sex differences in autophagy have been proposed to explain disparities between sexes in features of numerous pathological conditions including cardiovascular disease, cancers, and neurogenerative disease (30). While evidence that sex differences in autophagy influences the pathophysiology of sarcoidosis to the best of our knowledge has not been reported, it is tempting to speculate on a connection given the differences in clinical presentation, prognosis, and treatment response between males and females (31,32).

Obese and overweight individuals have an increased risk of sarcoidosis (33,34). Three studies in the United States, and one study in Denmark, have demonstrated increased risk of sarcoidosis in obese compared with nonobese patients with risk estimates ranging from 1.42 to 3.59 (35). mTORC1 activity is chronically upregulated during obesity (36,37). Along the same vein, tsc1 and tsc2 inactivation and constitutive mTORC1 activity has been shown to increase glycolytic flux, the oxidative arm of the pentose phosphate pathway, and *de novo* lipogenesis in mice models. This pathway is mediated by mTORC1-directed upregulation of hypoxia-inducible factor 1 (HIF1), a growth factor known to be implicated in cell growth and proliferation(38). Since obese individuals have excessive mTORC activation, basal autophagy may

be insufficient or inadequate (39). Collectively, these observations support the postulate that defects in autophagy play a role in sarcoidosis pathogenesis.

Targeting autophagy pathways in the clinical management of sarcoidosis

Glucocorticoids, a first line therapy for sarcoidosis, carry an unfavorable dose dependent and cumulative side-effect profile. Thus, several steroid-sparing alternatives are frequently used with variable degrees of supporting evidence, including anti-metabolites (methotrexate, azathioprine, leflunomide, mycophenolate mofetil), TNF inhibitors, and rituximab (40,41). Other, newer immunomodulatory therapies have been given to patients with sarcoidosis, with evidence limited mostly to case series and case reports. Several of these agents target the autophagy pathway either directly, or indirectly and have shown promising initial results (Figure 1d).

As mentioned above, direct pharmacological mTOR inhibition resulted in the resolution of granulomas in the murine model of sarcoidosis (20). In human studies, treatment of pulmonary sarcoidosis with mTOR inhibitors has been described in two case reports. The first describes a patient who developed sarcoidosis following liver transplantation for HCV-related cirrhosis. His transplant immunosuppression was changed from cyclosporine to the mTOR inhibitor sirolimus, after which he had histopathologic and radiologic resolution of sarcoidosis at two years of follow-up (42). The second study describes a sarcoidosis patient with recurrent symptoms despite high dose corticosteroid therapy. He then pursued off-label treatment with 2 mg of sirolimus daily for 10 months with resolution of his symptoms and significant radiologic improvement of pulmonary infiltrates (43).

Additionally, randomized controlled studies have been performed evaluating sirolimus for use in the treatment of non-infectious uveitis of all causes. Albeit in small proportions, sarcoidosis as an underlying cause for uveitis was represented in each of the trials (13% in SAVE 2013 and one year outcomes of SAVE 2013, 5% in SAVE 2016, and 8.4% in SAKURA) (44–47) reviewed in a meta-analysis showing encouraging results with 38% and 50% improvement in inflammation (measured by vitreous haze) and a 62.% and 57% improvement in visual acuity at 6 and 12 months, respectively (48).

Drugs targeting upstream activators of mTOR have also shown clinical efficacy in sarcoidosis. Having previously demonstrated that serum amyloid A (SAA), which is highly upregulated by IL-6, is increased in the serum of patients with sarcoidosis and is concentrated inside of sarcoidosis granulomas (49), investigators evaluated the therapeutic effect of IL-6 receptor inhibition (50). The four patients whose disease was unresponsive to several steroid-sparing agents who then received either monthly or biweekly infusions of the interleukin-6 (IL-6) inhibitor tocilizumab had improvement in their symptoms and organ function with a corresponding decrease in corticosteroid dosing. Since IL-6 activates mTORC1 via Janus tyrosine Kinase (JAK), it is conceivable that IL-6 inhibition stimulates autophagy that in turn, would increases clearance of SAA aggregates and lead to improved outcomes in sarcoidosis. Another case study of clinical and histologic remission of refractory cutaneous sarcoidosis using treatment with the JAK-STAT inhibitor tofacitinib, confirmed with GSEA down-regulation of mTORC1 pathway following therapy (51). Together, these studies support the existence of an IL-6-JAK-mTOR pathway of autophagy inhibition in the pathogenesis of sarcoidosis.

Conclusions

There is a growing body of evidence to suggest that dysregulation in cellular autophagy plays a fundamental role in the pathogenesis of sarcoidosis. This evidence includes studies in sarcoidosis patients that show evidence of impaired autophagy in affected tissues, and improved resolution of granulomatous inflammation in a murine model of sarcoidosis upon activation of autophagy completion. Advances in proteomic and genomic analysis in sarcoidosis led to identification of abnormalities in multiple signaling molecules and pathways that control cellular autophagy, most of which are anchored on the mTORC1 signaling pathway. Finally, several reports have shown efficacy of drugs that can dampen mTOR activation. Collectively, this evidence supports a central role for dysregulated autophagy in the pathogenesis of sarcoidosis.

Future directions

Identification of specific defects in autophagy pathways in sarcoidosis patients using exome and

transcriptome sequencing on samples obtained at the time of tissue diagnosis will be needed to reveal the extent and distribution of defects in autophagy pathways and correlate them with disease severity and outcomes. Such studies have the potential for establishing new biomarkers for disease risk, activity, and progression as well as personalize potential therapeutic options targeting autophagy pathways like mTORC1. Candidate drugs with direct or indirect mTOR inhibitory activity, and other approaches to optimize cellular autophagy will require investigations in larger randomized controlled studies of therapeutic effectiveness.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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