

Autophagy in the pathogenesis of ankylosing spondylitis

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Abstract The pathogenesis of ankylosing spondylitis (AS) is not well understood, and treatment options have met with limited success. Autophagy is a highly conserved mechanism of controlled digestion of damaged organelles within a cell. It helps in the maintenance of cellular homeostasis. The process of autophagy requires the formation of an isolation membrane. They form double-membraned vesicles called “autophagosomes” that engulf a portion of the cytoplasm. Beyond the role in maintenance of cellular homeostasis, autophagy has been demonstrated as one of the most remarkable tools employed by the host cellular defense against bacteria invasion. Autophagy also affects the immune system and thus is implicated in several rheumatic disease processes. In this article, we explore the potential role of autophagy in the pathogenesis of AS.

Keywords ATG16L1 · Autophagy · Inflammation · LRRK2 · Pathogenesis · Spondyloarthritis

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory arthritis affecting young men and women, resulting in significant disability and impact on quality of life. AS manifests

predominantly with severe back pain due to spinal arthritis and with peripheral joint involvement in a proportion of patients [1]. The pathogenesis of AS is not well understood, and treatment options have met with limited success. Novel pathogenic mechanisms are being investigated to better understand the pathogenesis of AS and recently autophagy has been identified as a potential player. Autophagy is a highly conserved mechanism, from yeast to mammals, of controlled digestion of damaged organelles within a cell essential for the maintenance of cellular homeostasis [2]. Under nutrient depletion conditions, cells recycle intracellular energy resources and under stress conditions cytotoxic proteins and damaged organelles are removed through autophagy [2].

Types of autophagy

Three different types of autophagy, differing in their mechanisms and functions, have been described [3]: (i) macroautophagy (in which the entire regions of cytosol are sequestered in vesicular compartments), (ii) microautophagy (mediated by direct lysosomal engulfment of cytoplasmic components), and (iii) chaperone-mediated autophagy (that is exclusively dedicated to degradation of soluble proteins). Macroautophagy is the most prevalent autophagy mechanism and is referred to as “autophagy” in this review.

Mechanism of autophagy

The process of autophagy requires the formation of isolation membranes (phagophores) that elongate engulfing a portion of the cytoplasm. They form double-membraned vesicles called “autophagosomes.” The formation of autophagosomes takes place in four major steps: initiation, nucleation,

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elongation, and closure. Each step is strictly regulated by proteins encoded by autophagy-related genes (*ATGs*) [3].

The initiation of autophagy takes place through the activation of the unc-51-like kinase 1 (Ulk1) complex. This Ulk complex is formed by the Ulk1 Ser/Thr protein kinase that mediates the phosphorylation of Atg13 and FIP200 and is essential for activating autophagy [4]. This is followed by phagophore nucleation that requires the Beclin1/hVps34 complex, consisting of Beclin1, hVps34, hVps15, and Atg14 [5]. Subsequently, autophagosome elongation and closure happens that requires two ubiquitin-like conjugation systems: Atg12 and light chain 3 (LC3) [6]. The cytosolic isoform of LC3 (LC3-I) is conjugated to phosphatidylethanolamine through two consecutive ubiquitination-like reactions. Atg7 and Atg3 are ubiquitin-like modifier-activating enzymes. The first step of LC3-I modification is catalyzed by the E1-like enzyme Atg7 followed by the E2-like enzyme Atg3, forming LC3-II [6]. LC3-II formation is used as a marker for autophagosomes in studies. Finally, the autophagosomes merge with lysosomes to form autolysosomes and lysosome enzymes can degrade the macromolecules engulfed by autophagosomes [6].

Autophagy in health and disease

Beyond the role in maintenance of cellular homeostasis, autophagy has been demonstrated as one of the most remarkable tools employed by the host cellular defense against bacteria invasion [7]. However, several pathogens can manipulate autophagy using molecular mechanisms as a strategy to establish persistent infection. Modulation of autophagy by specific intestinal microorganisms is observed in the gut of Crohn's disease (CD) patients [8], in which polymorphisms in autophagy genes, such as *ATG16L1* and *IRGM*, are associated with the incidence of intestinal inflammation [9]. In particular, a single-nucleotide polymorphism (SNP) encoding a missense variant in the autophagy gene *ATG16L1* (rs2241880, Thr300Ala) is strongly associated with CD [9]. The T300A variant significantly increases Atg16L1 sensitization to caspase-3-mediated processing, leading to accelerated degradation of Atg16L1 and impaired autophagy that predisposes individuals to CD [10].

Autophagy also affects the immune system and thus is implicated in several rheumatic disease processes. Autophagy can affect the thymic T cell selection process, antigen processing, cross presentation, cell survival, and even cytokine responses of immune cells. Thus, autophagy is of paramount importance to the immune system and relevant in the pathogenesis of rheumatic diseases. During thymic education, T cell gets exposed to MHC-peptide complexes on thymic epithelial cells. Only T cells that recognize peptides on self-MHC and are self-tolerant gets approval for further

development. Autophagosomes with cellular components can gain access to the MHC-II pathway and thus help present endogenous peptides to the developing T cells helping in developing self-tolerance [11, 12].

Autophagy in rheumatic diseases

Autophagy has been linked to several rheumatic diseases. SNPs in or around the autophagy genes *ATG5*, *ATG7* and *IRGM* have been found to be associated with SLE in genome wide association studies (GWAS) [13]. Autophagy is upregulated in peripheral blood T cells and splenic B cells of lupus-prone mice, and autophagy could contribute to auto-reactive T and B cell survival in lupus patients [14].

Compared to healthy control skin samples, systemic sclerosis patients have higher levels of autophagy in punch biopsies. However, these reports seem to be at odds with the observation that decreased autophagy increases fibrosis. GWAS studies in systemic sclerosis have identified associations with *ATG5* [15]. Caveolin 1 is a scaffolding intracellular protein that regulates autophagy [16]. Caveolin 1-deficient mice show evidence of systemic sclerosis like changes in the skin as well as develop pulmonary fibrosis [17].

Compared to normal control tissues, in both RA and OA, autophagy is upregulated [18]. RA synovial fibroblasts are resistant to apoptosis due to upregulation of autophagy [19, 20] and interestingly autophagy could contribute to methotrexate treatment resistance. Autophagy can activate osteoclastogenesis [21, 22]. Osteoclasts mediate erosive disease in RA. Interestingly, autophagosomes in antigen-presenting cells have peptidyl arginine deiminase activity (PADI) [23]. PADI converts arginine to citrulline resulting in the generation of citrullinated peptides. Autophagy is important for the presentation of citrullinated peptides but not other peptides by APCs [24]. Thus, a cardinal immune response in RA pathogenesis is mediated by autophagy.

Autophagy could also contribute to the immunopathogenesis of pSS. Autophagy is in fact upregulated in T cells from salivary glands but not from peripheral blood of primary Sjogren's Syndrome (pSS) patients, and it is correlated with disease activity and damage indices [25]. In pSS, autophagy seems to be also correlated with the local expression of the pro-inflammatory cytokines IL-21 and IL-23p19 [25]. Endoplasmic reticulum stress causes autophagy and apoptosis in salivary glands epithelial cells leading to cell surface and apoptotic bleb relocalization of Ro/SSA and La/SSB autoantigens [26].

Autophagy in ankylosing spondylitis

Despite the strong association between AS and inflammatory bowel diseases (IBD) and the high prevalence of

subclinical gut inflammation in AS patients, no association with autophagy genes was found in GWAS studies in AS [27]. A recent GWAS identified that anthrax toxin receptor 2 (ANTXR2), a gene potentially affect new bone formation, was one of the risk loci for AS [28]. In the peripheral blood from AS patients, ANTXR2 is downregulated along with the upregulation of miR-124. Intriguingly, the micro RNA miR-124 targets ANTXR2 and overexpression of miR-124 in Jurkat cells notably inhibits ANTXR2 expression, promoting JNK activation, and induces autophagy [29]. Another report on AS genetics suggested borderline significant association with the *LRRK2* gene [30]. *LRRK2* is closely linked to autophagy with varying reports suggesting *LRRK2* activates or negatively regulates autophagy [31, 32]. However, this association has not been validated in other studies.

Autophagy seems to be differentially modulated in AS patients with expression differences between tissues as well as different modulation of autophagy pathways within the same tissue. In the inflamed gut, autophagy, but not CMA, is significantly upregulated. This is especially true in the context of specialized epithelial cells located at the bottom of intestinal crypts, namely, Paneth cells (PC), and among infiltrating mononuclear cells. Sequestosome 1 (SQSTM1), an ubiquitin-binding protein that decreases when autophagy is induced, was not detectable in the gut of AS patients with chronic gut inflammation. Inhibition of autophagy and CMA (with 3-MA and anisomycin) was sufficient, in the presence of LPS, to increase the mRNA levels of IL-23p19 in the LPMCs isolated from the gut of AS patients, at the same time inducing IL-23p19 release. Interestingly, CMA was significantly downregulated in AS and negatively correlated with IL-23 levels, suggesting a role specifically for CMA in modulating IL-23 response [33]. The increase of IL-23p19 with inhibition of autophagy could be related to the ability of autophagosomes to sequester and target pro-inflammatory cytokines for degradation [34].

Unlike in the gut, a recent report indicates that there were no differences in the expression of autophagy-related genes in the synovial tissues of patients with spondyloarthritis [35]. In PBMCs isolated from AS patients, there was decreased expression of ATG16L1, IRGM, and HSP90AA1 compared to healthy controls, with no significant modulation of MAP1LC3A, ATG5, and HSPA8 expression. These findings suggest that autophagy activation in patients with AS is a tissue-specific phenomenon with an important role reserved for the gut. In this regard, autophagy has been demonstrated to play an important role in the innate immune response against intestinal bacteria, and its expression seems to be potentially modulated by specific gut microbiota. The presence of dysbiosis in the gut of AS patients has been recently proven [36]. Gut dysbiosis might result in active stimulation of the

innate immune system in AS patients, as indirectly suggested by the activation of PC in producing their antimicrobial products (lysozyme, phospholipase A2, and defensin 5) [37].

Another function of autophagy is connected to its ability to target improperly folded proteins for degradation in close connection with the endoplasmic reticulum stress response known as the unfolded protein response (UPR). Misfolding of HLA-B27, which can be defined as the propensity of HLA-B27 molecules to oligomerize and form complexes in the endoplasmic reticulum (ER) with the chaperone BiP (HSPA5/GRP78), occurs in AS patients. Unlike transgenic rats where HLA-B27 is overexpressed and UPR activation is prominent, accumulation of misfolded heavy chains in AS does not activate the unfolded protein response. In *in vitro* studies, autophagy suppression induces a significant increase in free heavy chains expression that was paralleled by a significant increase in UPR markers. The inability to demonstrate UPR in some *in vivo* studies of AS patients could be due to compensation by excess autophagy. Autophagy and UPR regulate each other and perturbations of this fine balance can influence the pathogenesis of AS.

Conclusions

Autophagy is an active cellular process that can influence the immune system in multiple ways. Changes in autophagy have been linked to several diseases. Autophagy could be a critical missing link in the pathogenesis of AS. Further assessment of autophagy in studies on AS pathogenesis is warranted.

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