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Mini-review

Molecular crosstalk between the proteasome, aggresomes and autophagy: Translational potential and clinical implications

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ABSTRACT

Targeting the ubiquitin+proteasome protein degradation pathway with the therapeutic agent bortezomib has significantly improved the survival of cancer patients but drug resistance inevitably develops. Aggresomes and the autophagy pathway serve as compensatory protein-clearance mechanisms that eradicate potentially toxic proteins to promote resistance to proteasome inhibitors and, hence, tumor survival. Preclinical evidence has emerged to demonstrate active crosstalk between these protein degradation pathways and has revealed novel therapeutic targets and strategies. Translational research and clinical trials are now focused on these pathways to prevent the emergence of drug resistance, enhance apoptosis and further improve the survival of cancer patients.

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1. Introduction

Exquisite regulation of the cell proteome ensures viability through a network of factors that mediate the expression, folding and transport of newly synthesized proteins coupled to the degradation of short-lived, misfolded, mutant and aggregated proteins [1,2]. Cells maintain a healthy state of self-renewal through the coordinated synthesis and degradation of intracellular proteins as demonstrated by deregulation of protein homeostasis that leads to neurodegenerative diseases and cancer [3]. Whereas *de novo* protein synthesis is a comparatively slow process, proteins are rapidly degraded at a rate compatible with the control of cell cycle transitions and the induction of cell death [4]. Protein degradation has been established as a major effector that governs the level of individual proteins and requires the coordinated efforts of three interconnected pathways: (1) the molecular chaperone machinery that utilizes heat shock proteins (Hsps) to assist in the efficient folding and translocation of polypeptides and importantly, also functions in the selection elimination of certain proteins following stress or mutation; (2) the ubiquitin (Ub) proteasome system (UPS) which is a highly complex network that controls the levels of short-lived proteins and functions to eliminate misfolded and denatured proteins [4]; and (3) aggresomes that sequester and deliver toxic protein aggregates for eradication either alone or in combination with autophagy [5].

Bortezomib (Velcade®, Millennium Pharmaceuticals) is a reversible proteasome inhibitor that has demonstrated potent *in vitro* antitumor activity either as a single agent or in combination with numerous cytotoxic agents against a broad spectrum of hematological and solid tumor types [6–8]. In preclinical studies, bortezomib induced apoptosis, sensitized cells to chemo- and radiotherapy and inhibited tumor growth in murine xenograft models [6–9]. Proteasome inhibition has been translated to the clinic for the treatment of certain hematologic malignancies with a significant improvement in overall survival (OS) [10]. Although

Abbreviations: Hsp, heat-shock protein; Ub, ubiquitin; UPS, ubiquitin+proteasome system; OS, overall survival; FDA, Federal Drug Administration; MM, Multiple Myeloma; MCL, Mantle Cell Lymphoma; ER, endoplasmic reticulum; UPR, unfolded protein response; MMCL, multiple myeloma cell lines; DRAM, damage-regulated autophagy modifier; CHIP, C-terminus of the Hsc-70-Interacting Protein; MTOC, microtubule organizing center; HDAC, histone deacetylase; UBA, Ub-association domain; SQSTM1, sequestome-1; BRCA, breast cancer gene; NBR1, neighbor of BRCA; LIR, LC3-interacting region; GABARAP, gamma-aminobutyric acid receptor-associated protein; LC3, light chain 3; ATG, autophagy-related gene; PB1, Phox/Bem 1p; UBL, ubiquitin-like protein; VCP, valosin-containing protein; VPS, vacuolar sorting protein; CQ, chloroquine; HCQ, hydroxychloroquine; 3-MA, 3-methyladenine; mTOR, mammalian/mechanistic target of rapamycin; SAHA, suberoylanilide hydroxamic acid; NF-κB, nuclear factor kappa B; PARP, poly ADP ribose polymerase; CML, chronic myelogenous leukemia; PTEN, phosphatase and tensin homolog; TSC1/2, tuberous sclerosis protein 1/2; HCC, hepatocellular carcinoma.

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bortezomib has received Federal Drug Administration (FDA) approval for the treatment of Multiple Myeloma (MM) and Mantle Cell Lymphoma (MCL), many patients do not respond to therapy [11,12]. In addition, those that do respond inevitably develop drug resistance as well as adverse toxicities such as peripheral neuropathy. Finally, bortezomib has not been successful in the treatment of solid tumors and, thus, novel agents either as monotherapy or in synergistic combination are needed to generate sustained clinical responses and to improve OS.

Intracellular proteins are targeted for proteasomal degradation by the covalent attachment of the highly conserved protein Ub in the form of a chain to a lysine residue on the protein targeted for degradation [4]. Molecular chaperones and Hsp's physically interact with targets to either promote efficient folding or to interact with additional factors that facilitate subsequent Ub chain attachment. Ub itself possesses seven lysines that can be used for the attachment of another Ub moiety and allow targets to be modified with different Ub chain types [13,14]. The consequences of polyubiquitylation are dependent upon the length and type of linkage used. The K48 Ub chain type is the most abundant and serves as the canonical signal for degradation by the 26S proteasome [15]. Since the proteasome is limited in its capacity to degrade membrane-associated, oligomeric and protein aggregates, other Ub chain types, e.g., K63, have been identified and function in a variety of non-proteasomal events such as protein trafficking, DNA repair and inflammation [16]. Importantly, K63 Ub chain types have been recently associated with target recognition by aggresomes and the autophagy pathway.

Since its discovery in the 1950s, autophagy has been thought to mediate the random, bulk clearance of long-lived cellular components including organelles and proteins [17]. Beginning in the 1970s, investigation of the UPS provided an understanding of the mechanism that controls the selective removal of individual soluble proteins. For many years, the UPS and autophagy were thought to function independently, fulfilled by distinct molecular effectors, separated subcellularly and to act on mutually exclusive substrates. However, recent findings point to an unforeseen link between these proteolytic pathways [18,19]. Certain proteins are removed through a process known as selective autophagy and point to not only molecular links with the UPS but active crosstalk between these systems in both normal and abnormal cells [20–22]. It is apparent that pharmacologics that perturb the flux of one pathway may affect the activity of the other. Under conditions in which the proteasome is inhibited or overloaded, autophagy may be upregulated to compensate for protein clearance to reduce the burden of UPS substrates. However, it is also possible that induction of autophagy yields unwanted consequences such as the removal of normally toxic proteins and leads to drug resistance to proteasome inhibition [23]. There is no consensus on the precise events responsible for autophagy induction but reported mechanisms include induction of the endoplasmic reticulum (ER) stress response and increased unfolded protein response (UPR) [reviewed extensively in 23]. It appears that MM cell lines (MMCLs) that harbor wildtype *p53* or mutant versions as well as *p53*-null cells are all equally sensitive to bortezomib. Also, the level of autophagy effectors and autophagosomes is increased by bortezomib treatment of MMCLs independent of *p53* status (unpublished observations). Therefore, multiple events such as AMPK activation, mTOR inhibition and DNA damage-regulated autophagy (DRAM) induction may be induced in a *p53*-independent fashion to upregulate autophagy [23].

2. Molecular linkage of the UPS with aggresomes and selective autophagy

Ubiquitylation may serve as a universal tag for degradation through either the UPS or autophagy, however, the precise type of ubiquitination recognized by each system appears to differ [22,23]. Biochemical, genetic and pharmacologic evidence indicates that the UPS is mechanistically linked with aggresomes and selective autophagy at multiple levels [22,23]. The clearance of toxic protein aggregates is achieved through aggresomes and a multi-step process known as "quality control" selective autophagy [24]. Misfolded proteins and translational mistakes are the inherent by-products of cellular biogenesis and accumulate through mutation, defects in the assembly of multimeric proteins, defective post-translational modification, nutritional deprivation or cellular stress. These perturbations lead to the accumulation of aggregates recognized by molecular chaperones, e.g., Hsp70, Hsp90, and E3 Ub ligases such as the C-terminus of the Hsc-70-Interacting Protein (CHIP) [25,26]. However, inhibition of the UPS also re-directs Ub-conjugates to non-proteasomal default pathways. Aggregated proteins accumulate in inclusion bodies known as aggresomes associated with histone deactylase 6 (HDAC6) and the microtubule organizing center (MTOC) located at the centriole [27]. Importantly, HDAC6-deficient cells are defective in the removal of protein aggregates and also cannot form large aggresomes [28,29]. Rather HDAC6-deficient cells form an increased number of micro-aggregates that are distributed throughout the cytoplasm [28,29]. While the precise molecular composition of aggresomes still is emerging, it appears that Hsps 27, 70 and 90, Ub, microtubules and HDAC6 are commonly detected components. The HDAC6 C-terminal region bears a Ub-association domain (UBA) that binds ubiquitinated proteins and is essential for HDAC6-mediated aggresome formation. Aggresomes are evident in neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, Lewy body dementia and Huntington's disease [30,31]. Although proteasomes and aggresomes have distinct structural compositions and substrate specificity, evidence suggests a mechanistic link and point to HDAC6 as a key player since cells that lack functional HDAC6 are unable to use aggresomes or autophagy to compensate for impaired UPS function.

Both the UPS and autophagy pathway possess substrate-binding molecules that recognize Ub-conjugated proteins. Autophagy refers to a number of related processes in which cellular cargo, e.g., proteins, mitochondria, organelles, microbes, are delivered to the lysosome either for clearance or for recycling [22–24]. This process involves the initial formation of double membrane-bound structures that surround the cargo to form an autophagosome that then serves as a vehicle for transport and delivery to the lysosome. Each specialized form of autophagy utilizes a distinct set of dedicated cargo receptors but the basic mechanism appears constant. Proteins that bear a Ub chain are recognized by selective autophagy cargo receptors that include p62/sequestome-1 (SQSTM1) and Neighbor of BRCA1 (NBR1) [32–36]. p62/SQSTM1 is a multifunctional adaptor protein implicated in cell signaling and differentiation that interacts with other proteins through a conserved N-terminal domain [37]. p62 possesses a zinc-finger, C-terminal UBA that binds both K48- and K63-linked Ub chains but displays a much higher affinity for K63 chains [38–40]. p62 has been implicated in both the UPS and autophagy systems. On one hand p62 recruits ubiquitinated protein aggregates to the autophagosome through two functional domains: the UBA that binds the Ub chain on cargo and the light chain 3 (LC3)-interacting region (LIR) domain which mediates direct interaction with the autophagy-specific proteins LC3 and the gamma-aminobutyric acid receptor-associated protein (GABARAP) also known as the autophagy-related gene 8 (ATG8) in yeast. On the other hand, p62 itself is a substrate for autophagic degradation, and inhibition of autophagy leads to the accumulation and aggregation of p62 through the Phox/Bem1p (PB1) domain. Elevated p62 may compete with other Ub-binding proteins involved in proteasomal degradation and may prevent ubiquitinated proteins from passing through