REVIEW

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Proteasomes and Proteasomal Gene Polymorphism in Association With Inflammation and Various Diseases

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Summary. *A proteasome, a multicatalytic protein complex, is a central particle of the ubiquitin-proteasome proteolytic pathway in all eukaryotic cells. Through the degradation of most intracellular proteins, proteasomes play a significant role in cell processes, such as cell cycle and division, posttranslational protein quality control, cell signaling, and apoptosis. Therefore, the ubiquitin-proteasome system is necessary to ensure the normal functioning of cells and an organism. The associations between alterations in the ubiquitin-proteasome pathway and the development of various autoimmune, neurodegenerative, inflammatory and other diseases in humans have been established. Moreover, the findings of some studies suggest that proteasomes may participate in the pathogenesis of asthma through the regulation of the nuclear factor kappa B signaling pathway. Recently, much attention has been given to the associations between genes encoding the proteasome and their polymorphism, and various diseases. Associations between some proteasomal genes and myocardial infarction, type 2 diabetes mellitus, and other diseases have already been established. However, the results are inconclusive or conflicting and need further clarification.*

Introduction

The proteasome is a central particle of the ubiquitin-proteasome proteolytic pathway, which is responsible for the degradation of almost all cell proteins (1). Short-lived normal proteins, which are not needed to the cell anymore, are degraded mostly via this pathway. These proteins are involved in cell cycle, apoptosis, gene transcription, cell signaling pathways, and endocytosis. Furthermore, the degradation of denatured, misfolded, mutated, damaged, or improperly translated proteins by proteasomes is an important step of posttranslational protein quality control (2, 3). Therefore, the normal activity of the ubiquitin-proteasome system is essential for the normal functioning of a cell (4).

Previous studies have shown that alterations in the ubiquitin-proteasome pathway can contribute to the development and progression of various human diseases, such as Parkinson disease, Alzheimer's disease, Angelman syndrome, ischemic heart disease, and other neurodegenerative, autoimmune, and inflammatory diseases (5–7).

Here we briefly review the recent data on the structure and functions of proteasomes, their impact on inflammation, and associations between proteasomal genes and various diseases.

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The Proteasome Proteolytic Pathway

The ubiquitin-proteasome proteolytic pathway starts from ubiquitin, as proteasomes cannot recognize a protein that is not targeted. Degradation of a protein via the ubiquitin-proteasome system (UPS) involves 2 successive steps (8): 1) covalent attachment of multiple ubiquitin molecules to the substrate; and 2) degradation of the tagged protein by the 26S proteasome and recycling of ubiquitin.

Ubiquitin is a highly evolutionarily conserved 76-residue polypeptide, which forms a compressed and almost globular structure. Selective attachment of ubiquitin to proteins is an initial signal for the degradation of targeted proteins. Conjugation of ubiquitin to the protein substrate proceeds via a 3-step cascade mechanism (Fig.). Initially, the ubiquitin-activating enzyme E1 activates a C-terminal Gly residue of ubiquitin in an ATP-requiring reaction. Then, the activated ubiquitin moiety is transferred to E2, an ubiquitin-carrier protein. Ubiquitin-protein ligase, a protein of the E3 family, recognizes and binds to the target protein. Then, the E2 protein transfers the activated ubiquitin moiety to the substrate, which is bound to E3. The activated ubiquitin moiety can be transferred either directly to the E3-bound substrate or via an E3 intermediate (3, 9, 10). This cycle repeats itself a few times until ubiquitin forms a sufficient-length chain by the consecutive transfer of activated ubiquitin moieties to an internal Lys residue of the previously conjugated

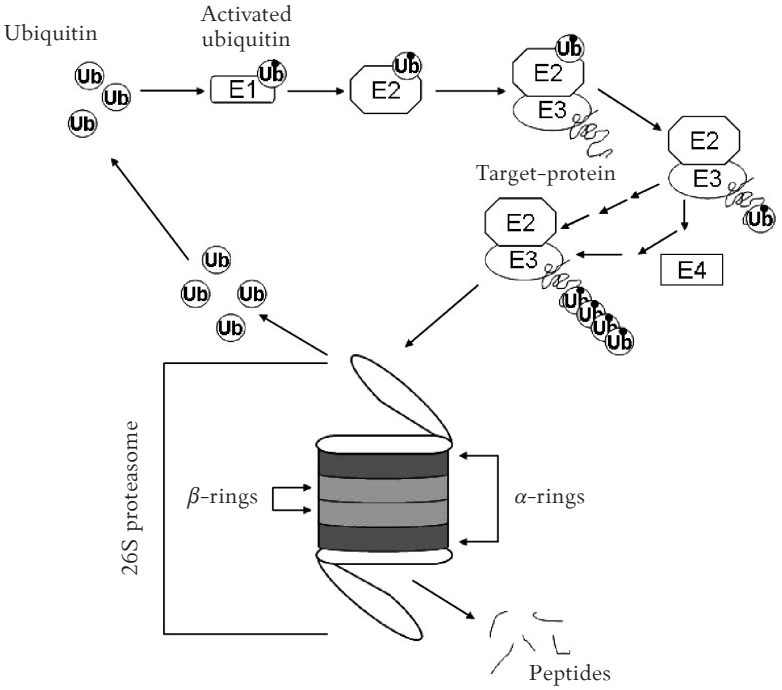

Fig. The ubiquitin-proteasome pathway

Ubiquitin is activated by the E1 enzyme and transferred to the E2 protein. A target-protein is recognized by E3, and then the E2 protein transfers the activated ubiquitin to the substrate, which is bound to E3. The cycle repeats itself until at least 4 of the activated ubiquitin molecules are attached to the target-protein. To tag some proteins with a multiubiquitin chain, the E4 protein is required. The ubiquitin chain is recognized by the 26S proteasome. The proteasome degrades the target-protein to short polypeptides and releases ubiquitin molecules, which can be activated again.

ubiquitin molecule. Ubiquitin moieties are linked to each other via isopeptide bonds and can form not only a straight, but also branched chain (11, 12). It has been reported that the shortest ubiquitin chain needed for efficient proteasomal targeting should be composed of 4 ubiquitin molecules (tetraubiquitin) (13). To target some proteasome substrates with a multiubiquitin chain, another protein E4, a chain elongation factor, is required (11).

The structure of the ubiquitin-conjugating and -activating system is hierarchical. A single E1 protein activates ubiquitin required for all modifications. It can transfer ubiquitin to several species of the E2 enzymes. More than 20 genes of E2 are discovered. Each E2 can transfer ubiquitin to one or several E3 proteins. There is a great diversity of E3s; about 1000 E3-encoding genes have been identified in the human genome. They can be subdivided into at least 2 groups according to the structure and class of signals they recognize. E3 proteins play a very important role in the ubiquitin-conjugating system, because they act as substrate-binding elements, which are responsible for the specificity and selectivity of the ubiquitin-proteasome system (10, 14).

However, ubiquitination does not always lead to the proteolysis of a targeted protein. Conjugation of ubiquitin may also be responsible for other functions such as the regulation of protein localization, functions, and their interactions. Furthermore, ubiquitination plays a very important role in histone modification (15).

Proteasome

The central part of the UPS is the 26S proteasome, also simply known as the proteasome. It is a protein complex that is expressed in the cytoplasm and nucleus of all eukaryotic cells (16). The 26S proteasome consists of 2 subcomplexes: a 20S catalytic core particle and a 19S regulatory particle (8, 17).

The 20S core particle has a barrel-shaped structure composed of 4 stacked rings, each formed by 7 different subunits. The 2 identical outer rings are composed of the α-type subunits and the 2 identical inner rings, of the β-type subunits. Therefore, the general structure of the proteasome complex is $\alpha_{1\text{-}7}\beta_{1\text{-}7}\beta_{1\text{-}7}\alpha_{1\text{-}7}$. The α-rings together with the 19S regulatory particles form a narrow channel through which only denatured proteins may pass. The β-rings, each of which contains 3 active sites, form a proteolytic chamber where proteins are degradated in a progressive manner. The proteolytic sites of the β-rings differ in their substrate specificity and proteolytic activity; therefore, they are named after enzymes that show similar activity or specificity: chymotrypsin-like, trypsin-like, and postglutamyl

peptide hydrolase-like (or caspase-like). During protein degradation in the proteolytic chamber, peptides of 3–25 amino acids in length are generated (16, 18, 19).

Both ends of the 20S particle are capped by the 19S particle. This particle is composed of 17 distinct subunits: 9 subunits form the base subcomplex and other 8 subunits form the lid subcomplex. The base, which binds to the core particle, has 4 non-ATPase and 6 ATPase subunits. The lid contains 8 non-ATPase subunits (10). The 19S regulatory particle has many functions. First, it recognizes ubiquitinated proteins and other potential substrates of the proteasome. Several ubiquitin-binding subunits of the 19S regulatory particle have been identified; however, their biological roles and mechanism of action have not been fully elucidated yet. A second function of this particle is to open an orifice in the α-ring that allows the substrate to enter into the proteolytic chamber. Since the folded protein does not fit to enter through the narrow proteasomal channel, unfolded substrates bind to the base, which possesses ATP energy-dependent chaperon-like activity. Then the 19S particle unfolds proteins and inserts them into the 20S core particle (19).

Thus, the UPS is a complex and very important cellular mechanism, which has not only proteolytic, but also nonproteolytic role in cells (20). Both of these roles are almost equally significant to the normal functioning of cells and disease development (21).

Proteasome System in Inflammation

During inflammation in cells, most of the inflammatory mediators are regulated by a transcription factor, nuclear factor kappa B (NF-κB). NF-κB is a heterodimeric transcription factor composed of the Rel family proteins. Three of the 5 family members – RelA (p65), c-Rel, and RelB – have a transcription-activating domain, while other 2 – p50 and p52 – can activate transcription only by heterodimerization with other Rel proteins (22, 23). The most common dimer is p50/p65 (14).

NF-κB is normally sequestered in the cytoplasm of nonstimulated cells where it is bound to the IκB proteins, masking its nuclear localization signal domain. When a cell is exposed to a variety of extracellular stimuli, such as environmental stress, cytokines, pathogen molecules, etc., the multisubunit IκB kinase complex (IKK) rapidly phosphorylates the IκB protein. Then, phosphorylated IκB is ubiquitinated and degraded in the proteasome, and the released NF-κB protein can translocate into the nucleus and initiate the gene transcription of proinflammatory factors: cytokines, adhesion molecules, chemokines, growth and cell cycle regulators, and others (24). Some cytokines can directly activate the NF-κB pathway, thus establishing a positive autoregulatory loop that can amplify the inflammatory response and increase the duration of chronic inflammation (25).

There are 2 main pathways of NF-κB activation: classical and alternative. The differences of these pathways are related to the IKK complex, which consists of 3 subunits. The most common form of this complex consists of the IKKα and IKKβ catalytic subunits and the IKKγ regulatory subunit. In the classical NF-κB signaling pathway, the activated IKK complex, predominantly acting through the IKKβ domain in an IKKγ-dependent manner, catalyzes the phosphorylation of IκB, polyubiquitination, and subsequent degradation by the 26S proteasome. The alternative NF-κB pathway is strictly IKKα-dependent and IKKβ- and IKKγ-independent. Both pathways are involved in the regulation of innate and adaptive immunity (26, 27).

Thus, NF-κB plays a very important role in the development and progression of inflammatory diseases. The activation of NF-κB pathway can promote proinflammatory processes and the development of chronic inflammatory disorders, but NF-κB can also act as an anti-inflammatory factor depending on the cell lineage and pathophysiological context (28, 29). Moreover, the mode of action of this pathway may change depending on regulatory factors. One of the main regulators is the UPS, which is involved in at least 3 distinct steps of the NF-κB signaling pathway (30).

p50 and p52 proteins in cells are synthesized as large inactive precursors (p105 and p100, respectively). To generate mature active proteins, the precursors are specifically and precisely degraded via the ubiquitin-proteasome system. The C-terminal domain is degraded, and an active protein is derived from the N-terminal domain (31). Therefore, primarily proteasomes are involved in the formation of an active NF-κB dimer.

An active dimmer of the NF-κB factor in cytoplasm is silenced by the IκB protein, which acts as an inhibitor covering the p65 part required for translocation to the nucleus. The cytoplasmic retention of the inhibited complex p50/p65/IκB is released upon kinase-mediated dual phosphorylation of IκB. Phosphorylated IκB is recognized by ubiquitin ligase and then polyubiquitinated and thereafter degraded by the proteasome. Once IκB has been degraded, the p50/p65 complex is able to translocate into the nucleus and initiate the inflammatory gene response program (14).

Moreover, the ubiquitin-proteasome system is involved in the activation of the IKK complex, which begins from the activation of tumor necrosis factor (TNF) receptor (29). Polyubiquitination of the RIP1 protein is a very important stage of this

signaling pathway. This protein promotes the activation of the IKK complex (32).

The ability of proteasomes to control the NF-κB activity was shown in studies on proteasomal inhibitors. Silencing of the NF-κB pathway through proteasomal inhibition has shown the promising results in the studies on various cell lines and small animal models with asthma (33, 34). Much attention has been given to proteasomal inhibitors not only as to inflammation suppressors, but also as potential drugs for anticancer therapy (35, 36). The clinical studies carried out with these inhibitors in subjects with cancer have also shown good results (37–39). Therefore, research on proteasomes and their inhibitors could provide new therapeutic agents for the treatment of various diseases, such as cancer, chronic inflammatory diseases, autoimmune diseases, multiple sclerosis, and other diseases, even stimulation of bone growth (40, 41).

Thus, by participating in the regulation of the NF-κB signaling pathway, proteasomes are also involved in the regulation of inflammation and asthma.

Association Between Proteasomal Genes and Various Diseases

The functioning of the ubiquitin-proteasome system and its participation in distinct cell signaling pathways is a complicated and not fully clarified process. However, given the already explained importance of proteasomes to normal cell functioning and relevance to human diseases, it is not surprising that interest in proteasome-coding genes is recently increasing. In the human genome, there are many genes that encode the proteins of the ubiquitin-proteasome system. In addition, the 26S proteasome is composed of 33 distinct subunits each encoded by different genes (42). A growing body of evidence shows that proteasome-encoding genes or their variants can have an impact on the development of human diseases.

Recently, much attention has been given to the *PSMA6* gene that encodes the proteasomal alpha subunit type 6 and is located in the chromosome 14q13.2 region. The *PSMA6* gene is one of the best evolutionary conserved genes of the alpha-family (43); therefore, even minor alterations in the translation of the *PSMA6* gene may have a huge impact on cell functions. There are studies that investigated associations between other proteosomal genes and certain diseases, but these studies are not as numerous as those on the *PSMA6* gene. Table summarizes the known associations between the proteasomal genes having polymorphism and various diseases.

Myocardial Infarction. Inflammation is considered a key factor in the pathogenesis of myocardial infarction (MI), and the polymorphism of the genes encoding inflammation regulators such as proteasomes may also play an important role in the development of this disease. In a study by Ozaki et al., a possible association between polymorphism of the *PSMA6* gene and MI in the Japanese population was investigated (47). The authors compared the genotype frequencies of 450 individuals with MI and 450 control individuals. They reported that one single nucleotide polymorphism (SNP) in the 5′ untranslated region of exon 1 of *PSMA6* (exon 1–8C/G, listed in the dbSNP database as rs1048990) was significantly associated with MI. It is thought that the G allele, which is considered a risk allele, might increase the expression of *PSMA6* leading to enhanced inflammation through the activation of the NF-κB protein (47). This study gave rise to many other studies, which were performed in different countries with different populations, and polymorphism of the proteasomal genes has gained increasing popularity among scientists. The study in a Chinese population also reported that the *PSMA6* variant rs1048990 could be a risk factor of MI and confirmed the association between polymorphism of the *PSMA6* gene and the development of MI (46). However, similar studies in other populations

Table. Known Associations Between Polymorphism of Proteasomal Genes and Diseases in Various Populations

Gene	Disease	Population	References
PSMA6	Myocardial infarction and cardiovascular diseases	Japanese, Caucasian (United Kingdom, South Italy), Chinese	(44–48)
	Type 2 diabetes mellitus	Latvian, Finnish, Botnian	(49–51)
	Coronary artery disease	Saudi Arabia	(52)
	Graves' disease	Latvian	(43)
	Juvenile idiopathic arthritis	Latvian	(53, 54)
PSMD9	Coronary artery diseases in type 2 diabetes	Italian	(38)
	Type 2 diabetes mellitus	Italian	(38)
PSMB4	Major depressive disorder	Mexican American	(55)
PSMB8	Insulin-dependent diabetes mellitus	Caucasian	(56)
	Pigeon breeder's hypersensitivity pneumonitis	Mexican	(57)
PSMB9	Insulin-dependent diabetes mellitus	Caucasian	(56)

showed controversial results. No associations between the *PSMA6* polymorphism and the risk of MI were shown in a United Kingdom population (51) and an Indian population (58); Bennet et al. concluded that the risk of rs1048990 polymorphism for MI was unlikely to be great in Western populations (45). Moreover, none of the single nucleotide polymorphisms of the *PSMA6* gene was found to be associated with coronary artery disease in a study carried out in Saudi Arabia (52) and coronary atherosclerosis and MI in Japan (59). Also large meta-analysis performed by Wang et al. suggested that the G allele of the rs1048990 polymorphism is a risk factor associated with increased susceptibility to coronary artery disease, but these associations vary in different ethnic populations (60). However, a borderline association of the rs1048990 with diastolic blood pressure was detected in the United Kingdom population (51), and recently, its association with susceptibility to ischemic stroke in both Caucasian and African Americans has been shown (48). Therefore, the question if the *PSMA6* gene can really have an impact on the development of MI is still open.

Diabetes Mellitus. In diabetes, proteasomes may play an important role through insulin degradation, internalization of insulin receptors, and inflammation as well. The susceptibility of the *PSMA6* polymorphism to type 2 diabetes mellitus was found in the Botnian (49), Finnish (50), and Latvian (51) populations. Moreover, it was shown that heterozygosity at the rs1048990 could be significant risk factor to type 2 diabetes mellitus in Latvians (51). Since diabetes mellitus is one of the major risk factors for the development of MI, a possible association between proteasomal genes and MI in type 2 diabetes mellitus has been suggested. The results of one Italian study in a Caucasian population showed that the *PSMA6* (-8 UTR C/G) polymorphism might contribute to MI susceptibility in type 2 diabetes mellitus (44). It is suggested that the enhanced transcription of the *PSMA6* gene in people with type 2 diabetes mellitus might contribute to an abnormal or exaggerated inflammatory response through an enhanced degradation of the inhibitory IκB proteins and thus increased NF-κB activation (44). Other proteasomal genes – proteasome subunit beta type 8 (*PSMB8* or *LMP7*) and proteasome subunit beta type 9 (*PSMB9* or *LMP2*) – located within the class II region of the major histocompatibility complex showed susceptibility to insulin-dependent diabetes mellitus (56).

Cancer. Genome wide association studies have identified the susceptibility locus for lung cancer to be present on chromosome 15. This locus 15q24-15q25.1 contains not only the genes of nicotinic acetylcholine receptor subunits (*CHRNA3* and *CHRNA5*), which could be good candidate genes for lung cancer, but also *PSMA4*, the proteasome alfa type subunit isoform 1 gene. This region was found to be strongly associated with the risk of lung cancer in the Caucasian and African American populations (61–63). Other in vitro study showed that the *PSMA4* gene played an important role in cancer cell proliferation (64). Moreover, it was reported that the *PSMA6* polymorphism (–8 UTR C/G) had a putative role as a survival factor in multiple myeloma, and the G allele could be considered a prognostic factor of survival (65). However, the studies exploring the associations between proteasomal genes and cancer are scarce, and more results are needed to confirm the role of proteasomal genes in the development of cancer.

Other Autoimmune Diseases. Although the main interest is given to the associations between proteasomal genes and MI along with diabetes mellitus, a few associations with other diseases were found. In the Latvian population, polymorphism of several intronic microsatellites within and upstream the *PSMA6* gene region was found to be associated with Grave's disease, an autoimmune thyroid disorder (43), and juvenile idiopathic arthritis (54). A borderline association between *PSMA6* -110C/A (rs2277460) and juvenile idiopathic arthritis was reported in the Latvian preliminary study (53). Genetic variations in *PSMB8* were shown to increase the risk to develop hypersensitivity pneumonitis, which is a lung inflammatory disorder caused by the inhalation of organic particles by a susceptible host (57). Moreover, the single nucleotide polymorphism in *PSMB4*, the proteasome beta subunit 4 gene located on chromosome 1, was found to be significantly associated with major depressive disorder in the Mexican American population (55). Recently, the point mutation in the *PSMB8* gene has been reported in Japanese patients with Nakajo-Nishimura syndrome. This mutation was shown to be a direct cause of this rare autoimmune human disease (66).

Asthma. Asthma is a chronic inflammatory disease of airways, which is characterized by reversible airway obstruction, bronchial hyperresponsiveness, and airway inflammation. The key pathological features involves infiltration of the airways by inflammatory and immune cells, structural damage of the bronchial epithelium, and mast cell degranulation (67). These pathological factors are regulated by inflammatory cells, predominantly eosinophils, lymphocytes, and neutrophils, which infiltrate the airways and release a myriad of proinflammatory substances including chemokines, cytokines, growth factors, and other peptides to further facilitate the development of disease (68). Previous findings suggest that proteasomes may participate in the pathogenesis of asthma through regulation of the

NF-κB signaling pathway. However, while searching in the PubMed database, we could not find any article on associations between proteasomal genes and asthma, but we hope that upcoming projects will bring some clearness in this field. The project "Proteasomal Gene Alleles as Risk Factors for Bronchial Asthma in Latvian, Lithuanian, and Taiwanese Populations" involves a study of 5 single nucleotide polymorphisms in 3 proteasomal genes (*PSMA6*, *PSMA3*, and *PSMC6*) to be evaluated in 3 different populations: Lithuanian, Latvian, and Taiwanese.

Although many studies have shown an importance of proteasomes and proteasomal genes in the development and progression of different pathologies, the role of the polymorphism of proteasome-coding genes in predisposing individuals to various diseases has not been fully understood, and the answers are still needed to be found.

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Statement of Conflict of Interest

The authors state no conflict of interest.

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