Ubiquitin, Proteasome, and Restenosis
A Brave New World for Cardiovascular Research
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In the present issue of Circulation, Meiners and his colleagues1 show that local application of a proteasome inhibitor resulted in significant inhibition of intimal hyperplasia in a balloon injury model in rat carotid artery. This report is of interest because it identifies a novel target for the treatment of restenosis and introduces a rapidly developing field in basic science to cardiovascular researchers.

Proteasomes are large intracellular protein complexes that specialize in degrading cellular proteins that are either unneeded or damaged.2–5 Inhibiting the activity of proteasome not only stops the ability of the cell to get rid of unwanted proteins, but also blocks most cellular processes critical for a cell’s survival. Inhibitors of proteasome activity have been used widely in basic research and more recently in anticancer clinical trials. It is not surprising that proteasome inhibitors can inhibit intimal hyperplasia because they generally have antiproliferative, antiinflammatory, and proapoptotic properties. In order to understand the inner workings of proteasome, one must become acquainted with a small protein called ubiquitin that serves as a molecular tag directing unneeded or damaged proteins to the proteasome for degradation.

Ubiquitin, a 76 amino acid protein, is highly conserved in evolution with only a 3 amino acid difference between yeast and human homologues. Most of the known function of ubiquitin resides in its ability to covalently attach to other proteins through the formation of an isopeptide bond attaching the C-terminal Glycine residue of ubiquitin to a Lysine residue of the target protein.2,4,5 A second ubiquitin then attaches to the first ubiquitin to build up a polyubiquitin chain, which is required for the tagged protein to be recognized by the proteasome. The process of attaching ubiquitin molecules to other proteins is termed ubiquitination; it occurs through a highly regulated biochemical process depicted in the Figure. First, ubiquitin is activated in an ATP-dependent process by the ubiquitin-activating enzyme (E1). The C-terminal Glycine residue of ubiquitin forms a thiol ester linkage with the active site Cysteine residue of E1. The activated ubiquitin moiety is then transferred to a ubiquitin-conjugation enzyme (E2) to form a second thiol ester linkage. There is only one E1 gene in mammalian cells but more than a dozen E2s, which are not sufficient to selectively modify thousands of cellular proteins at the precise timing and location. The fine specificity of regulated ubiquitination is achieved through precise engagement of a ubiquitin ligases (E3), selected from a large pool of E3s that belong to 4 different classes.4 Only the SCF type of ligase complex will be discussed in more detail in this editorial; interested readers can obtain more information from several excellent reviews.2,4

The SCF ligase complex is named because it is composed of Skp1, Cul-1, a F-box protein, and Rbx1.6,7 The Figure only depicts 3 components of the SCF ligase complex and shows that it connects E2 and the target protein together to allow for efficient transfer of ubiquitin to the target protein. In mammalian cells, over 50 F-box proteins have been identified that bind to Skp1 through the F-box motif and interact with target proteins in a phosphorylation-dependent manner.8 For example, tumor necrosis factor and interleukin-1 can activate a kinase cascade to phosphorylate IκBα on serine residues 32 and 36.9 Phosphorylated IκBα is then recognized by βTrCP, an F-box protein that specifically recognizes phosphorylated IκBα.10–12 The bound IκBα is then polyubiquitinated on lysine residue 21 and 22 to prepare for transfer to the proteasome for degradation. As IκBα is degraded, NF-κB is freed to translocate to the nucleus to participate in transcriptional regulation of multiple genes involved in inflammation.13 By blocking proteasome activity, IκBα cannot be degraded and NFκB remains in the cytosol. Thus, proteasome inhibitors can exert a potent antiinflammatory action by inactivating the signaling pathway of tumor necrosis factor and interleukin-1.

The core of the proteasome (20S) is made from 28 proteins that form 4 rings stacked to constitute a hollow cylinder.14 (see Figure) The outer rings (pink) are formed by 7 α subunits that are catalytically inactive; the inner rings (purple) are composed of 7 β subunits that have 3 distinct proteolytic activities: trypsin-like, chymotrypsin-like, and post-glutamyl peptidyl hydrolytic activities. Inside the hollow cylinder, proteases in the β subunits extend their blade to cut the target protein into smaller pieces (~8 amino acids in length). Amazingly, the processed peptide fragments can bind to the antigen-presenting groove of the class I major histocompatibility molecule to be recognized by the T-cell receptor.15 Thus, proteasome inhibitors also block antigen processing and T-cell activation. The proteasome is not complete without the caps (blue), which are formed by protein subunits involved in recognizing the polyubiquitin chains, unfolding the
target proteins, and releasing the ubiquitin tags for recycling. Together, the regulatory caps and the core cylinder form the so-called 26S proteasome.

The initial proteasome inhibitors, such as ALLM, were derivatives of calpain inhibitors; they inactivate the catalytically active β subunits of the proteasome but also inhibit calpains at higher concentrations.15 Meiners et al.1 used a more specific proteasome inhibitor, MG132, which is cell-permeable and inhibits the proteasome activity with a Ki of 4 nmol/L. They showed that MG132 inhibited cell proliferation, blocked activation of NFκB, and induced apoptosis in rat vascular smooth muscle cells. Furthermore, MG132 inhibited the degradation of c-jun, p21, and p53, which are known substrates of the ubiquitin/proteasome pathway. Armed with the in vitro results, the authors tested the effect of local application of MG132 in a rat balloon injury model and showed that neointimal hyperplasia was reduced significantly. They were able to demonstrate that the in vivo effect is due to reduced cell proliferation, decreased mononuclear cell recruitment, and induction of prolonged apoptosis. These results convincingly demonstrated that inhibition of proteasome activity is a potential therapeutic option in the treatment of restenosis.

It should be emphasized that the ubiquitin/proteasome system is not simply a “garbage disposal” system for the removal of damaged proteins. As shown in the studies reported by Meiners et al.,1 proteins involved in cell cycle progression (p21), signal transduction (IkBα), tumor oncogenesis (p53), and transcription regulation (c-Jun) are all regulated by the ubiquitin-dependent proteasome degradation pathway. It is not surprising that blocking the activity of the proteasome can inhibit restenosis, which is characterized by cell proliferation, inflammation, and dysregulated apoptosis. It is too early to say whether proteasome inhibitors could be used orally or intravenously to treat restenosis because of its potential systemic toxicity. It is conceivable, however, these inhibitors can be used to coat stent similar to the use of rapamycin in the treatment of restenosis.16 Efforts are underway to design inhibitors to block specific ligase activity. These specific inhibitors of ubiquitination will then allow for a more precise targeting of drug therapy.

In recent years, several ubiquitin-like proteins, such as Sentrin and NEDD8, were shown to modify other cellular proteins in a manner analogous to ubiquitination.17 Sentrin was originally discovered in a yeast 2-hybrid assay using the death domain of Fas as bait.18 Interestingly, Sentrin also binds to the death domain of tumor necrosis factor receptor-1. Overexpression of Sentrin can inhibit both anti-Fas and tumor necrosis factor–induced cell death.19 A large number of proteins have now been shown to be modified by Sentrin.20 It is clear that the Sentrin-modification pathway also play an important role in cell division, cell signaling, and tumorigenesis and will provide abundant targets for drug discovery. It should be noted that Sentrin-modified proteins are not degraded by the proteasome and may even compete with ubiquitin for the same Lysine residue of the target protein. Another ubiquitin-like protein, NEDD8, is structurally and functionally more related to ubiquitin.19 NEDD modifies Cul1 and may be required for SCF E3 ligase activity.20 Recently, we discovered a novel interferon-inducible protein, NUB1, which recruits NEDD8-conjugates to the proteasome for degradation, providing a direct link between these 2 systems.21,22 Thus, the ubiquitin frontier also intersects with other newly discovered ubiquitin-like territories that will provide cardiovascular scientists a brave new world for research and discovery.

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References


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