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Deubiquitinase inhibition as a cancer therapeutic strategy

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ABSTRACT

The ubiquitin proteasome system (UPS) is the main system for controlled protein degradation and a key regulator of fundamental cellular processes. The dependency of cancer cells on a functioning UPS has made this an attractive target for development of drugs that show selectivity for tumor cells. Deubiquitinases (DUBs, ubiquitin isopeptidases) are components of the UPS that catalyze the removal of ubiquitin moieties from target proteins or polyubiquitin chains, resulting in altered signaling or changes in protein stability. A number of DUBs regulate processes associated with cell proliferation and apoptosis, and as such represent candidate targets for cancer therapeutics. The majority of DUBs are cysteine proteases and are likely to be more "druggable" than E3 ligases. Cysteine residues in the active sites of DUBs are expected to be reactive to various electrophiles. Various compounds containing α,β-unsaturated ketones have indeed been demonstrated to inhibit cellular DUB activity. Inhibition of proteasomal cysteine DUB enzymes (i.e. USP14 and UCHL5) can be predicted to be particularly cytotoxic to cancer cells as it leads to blocking of proteasome function and accumulation of proteasomal substrates. We here provide an overall review of DUBs relevant to cancer and of various small molecules which have been demonstrated to inhibit DUB activity.

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1. Introduction

Proteins are vital to the structure and function of cells, and as such the regulated control of protein turnover is a fundamental aspect of cellular metabolism. ~30% of newly synthesized proteins in mammalian cells are rapidly degraded with a half-life of <10 min (Schubert et al., 2000). Such a high rate of protein turnover requires a specialized system for the controlled and selective degradation of unwanted proteins. The ubiquitin–proteasome system (UPS) has emerged as a key regulator of protein function and stability. At its most simple level the UPS is composed of a tagging factor in the form of the small molecule ubiquitin which marks unwanted or damaged proteins for degradation, and the proteasome, a large molecular shredder that breaks down proteins into smaller peptides for use in other anabolic processes. More than 80% of cellular proteins are degraded by the UPS, high-lighting the importance of this pathway in the regulation of multiple cellular processes (Rock et al., 1994). The multifaceted role of the UPS includes the degradation of misfolded and damaged proteins, cell cycle regulators, oncogene and tumor suppressor proteins, as well as the regulation of

Abbreviations: 19S RP, 19S regulatory particle; 20S CP, 20S core particle; ASK1, apoptosis signaling kinase 1; BRCA1, breast cancer type 1 susceptibility protein; Eer1, eeyarestatin 1; ERAD, ER-associated protein degradation; GA, gambogic acid; JAMM, JAB1/MPN/MOV34 metalloenzyme; MAPK, mitogen activated protein kinase; NFκB, nuclear factor kappa B; PCNA, proliferating cell nuclear antigen; PG, prostaglandin; Rpn11/POH1, regulatory particle subunit 11/pad one homolog-1; TLS, trans-lesions synthesis of DNA; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; UCH, ubiquitin carboxyl-terminal hydrolase; UPS, ubiquitin proteasome system; USP, ubiquitin specific peptidase.

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antigen processing and control of transcription factor activity (Coux et al., 1996; Hershko & Ciechanover, 1998). Considering the diversity of UPS substrates it is no surprise that this pathway has been implicated in the pathogenesis of many human diseases such as neurodegenerative disorders, viral diseases and cancer (Ciechanover et al., 2000).

The process of ubiquitination is a multi-step process ultimately leading to the covalent modification of a protein substrate with the small molecule ubiquitin. Ubiquitin is a highly conserved 76 amino acid protein that undergoes covalent attachment via an isopeptide bond between the carboxy glycine residue (G76) of ubiquitin to the ε-amino groups of lysine residues in target proteins. The process of ubiquitination is dependent on the consecutive activity of three distinct enzymes, Ub-activating (E1), Ub-conjugating (E2) and Ub-ligating (E3) (Fig. 1). In the first step, ubiquitin is activated by the E1 enzyme in the presence of ATP, forming a thioester bond between the carboxy-terminal glycine residue of ubiquitin and the active site cysteine of the E1 enzyme. Once activated, ubiquitin is transferred from E1 to a cysteine residue of one of the 30–40 E2 ubiquitin carrier proteins. Substrate specificity is conferred by E3 ligases, which bind target substrates and co-ordinate the covalent attachment of ubiquitin. Two distinct families of E3 ligases exist, the HECT domain family that receives ubiquitin from the E2 ligase forming an ubiquitin-E3 intermediate, and the RING finger family of E3 ligases that form a molecular bridge between the E2 ligase and target proteins. There are >500 E3 ligases in cells, making them the main specificity factor in the UPS (Hershko et al., 1983; Voges et al., 1999; Pickart & Eddins, 2004).

There are three different classes of ubiquitination: i) mono-ubiquitination where a single ubiquitin is attached, ii) multi-ubiquitination or poly-monoubiquitination where several single ubiquitin moieties are attached, and iii) poly-ubiquitination where substrates are tagged with polyubiquitin chains (Jentsch & Schlenker, 1995; Hicke, 2001; Di Fiore et al., 2003; Haglund et al., 2003; Haglund & Dikic, 2005; Ye & Rape, 2009; Lander et al., 2012). In addition to the three different classes of ubiquitination, a ubiquitin code exists whereby the type of linkages between ubiquitin monomers determines function. Ubiquitin contains seven lysine residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 and Lys63), any of which can serve as sites for the covalent attachment of other ubiquitin molecules. The nature of the linkages within the polyubiquitin chain has consequences in determining the fate of the conjoined protein. In general, proteins tagged with Lys48-linked polyubiquitin chains are destined for proteasomal degradation (Chau et al., 1989; Hershko & Ciechanover, 1998), whereas modifications involving Lys63-linked chains are more typically associated with non-proteasomal roles such as DNA repair, DNA replication and signal transduction (Haglund & Dikic, 2005). Other linkage types are generally less well characterized, although reports have shown that polyubiquitin chains linked through Lys6, Lys11, Lys27, Lys29, or Lys33 can target proteins for proteasome-mediated degradation (Xu et al., 2009). Even Lys63 chains, which are more traditionally implicated in signaling, can target the attached protein to the proteasome for degradation (Saeki et al., 2009).

The process of ubiquitination is highly dynamic and can be reversed by the action of specialized enzymes known as deubiquitinases (DUBs). DUBs oppose the action of the E3 ligases by cleaving the isopeptide bond between lysine residues on target proteins and the C-terminal glycine of ubiquitin. Analysis of the human genome shows the presence of ~80

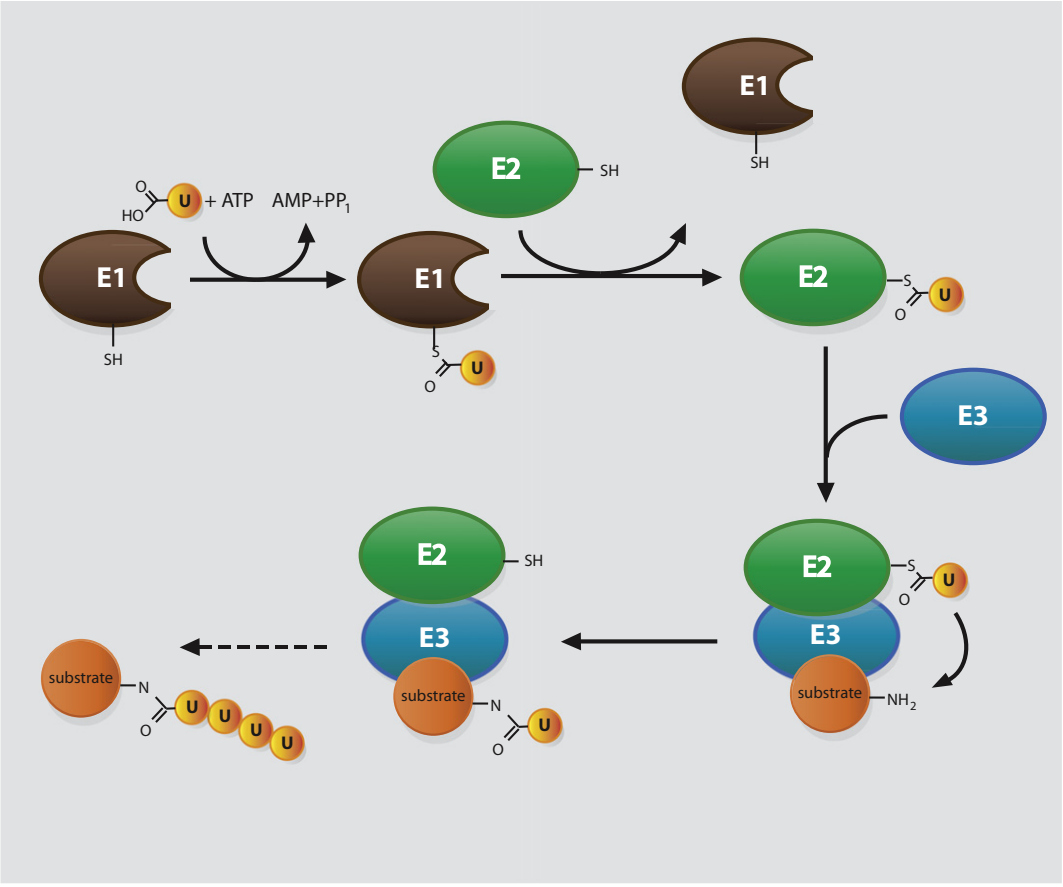

Fig. 1. Ubiquitination of proteins. Proteins are targeted for degradation by the addition of ubiquitin chains to lysine residues by a process that involves three enzymes. Ubiquitin is activated by a ubiquitin-activating enzyme (known as E1) and transferred to a cysteine residue of a ubiquitin carrier protein (known as E2). E3 ligases bind target substrates and co-ordinate the covalent attachment of ubiquitin. The existence of a large number (>500) of E3 ligases makes them the main specificity factor in the UPS. Target proteins may be monoubiquitinated or, as in this example, polyubiquitinated. A target protein must be tagged with at least four ubiquitin monomers (forming a polyubiquitin chain) to be recognized by the proteasome.