Development of Protacs to Target Cancer-promoting Proteins for Ubiquitination and Degradation*

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The proteome contains hundreds of proteins that in theory could be excellent therapeutic targets for the treatment of human diseases. However, many of these proteins are from functional classes that have never been validated as viable candidates for the development of small molecule inhibitors. Thus, to exploit fully the potential of the Human Genome Project to advance human medicine, there is a need to develop generic methods of inhibiting protein activity that do not rely on the target protein’s function. We previously demonstrated that a normally stable protein, methionine aminopeptidase-2 or MetAP-2, could be artificially targeted to an Skp1-Cullin-F-box ubiquitin ligase complex for ubiquitination and degradation through a chimeric bridging molecule or Protac (proteolysis targeting chimeric molecule). This Protac consisted of an SCF\(^{β-TRCP}\)-binding phosphopeptide derived from IxB\(\alpha\) linked to ovalicin, which covalently binds MetAP-2. In this study, we employed this approach to target two different proteins, the estrogen (ER) and androgen (AR) receptors, which have been implicated in the progression of breast and prostate cancer, respectively. We show here that an estradiol-based Protac can enforce the ubiquitination and degradation of the \(α\) isoform of ER \textit{in vitro}, and a dihydroxytestosterone-based Protac introduced into cells promotes the rapid disappearance of AR in a proteasome-dependent manner. Future improvements to this technology may yield a general approach to treat a number of human diseases, including cancer.

\textit{Molecular & Cellular Proteomics} 2:1350–1358, 2003.

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One of the major pathways to regulate protein turnover is ubiquitin-dependent proteolysis. Post-translational modification of proteins with ubiquitin occurs through the activities of ubiquitin activating enzyme (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3), which act sequentially to catalyze the attachment of ubiquitin to lysine residues in an energy-dependent manner (1, 2). Among the hundreds of E3s encoded within the human genome, the Skp1-Cullin-F-box (SCF) ubiquitin ligases comprise a heterotetrameric group of proteins consisting of Skp-1, Cul1, a RING-H2 protein Hrt1 (also known as Roc1 or Rbx1), and an F-box protein (1, 3). The mammalian F-box protein \(β\)-transducin repeat-containing protein (\(β\)-TRCP) of SCF\(^{β-TRCP}\) binds IxB\(\alpha\), the negative regulator of NF-\(κ\)B, and promotes its ubiquitination and degradation (4). A 10-aa phosphopeptide segment of IxB\(\alpha\) is both necessary and sufficient to mediate its binding to SCF\(^{β-TRCP}\) and subsequent ubiquitination and degradation (4).

There is a pressing unmet need to develop effective drugs to treat cancer and other diseases that afflict humans. The recent completion of the human genome sequence coupled with basic studies in molecular and cellular biology have revealed hundreds to thousands of proteins that could conceivably serve as targets for rational drug therapy. Unfortunately, many of these protein targets are not considered to be readily “drugable,” in that they are not enzymes and it is not obvious how to inhibit their function with small molecule drugs. Thus, it would be valuable to have a generic method that would enable specific and efficacious inhibition of any desired protein target, regardless of its biochemical function. Short interfering RNA (siRNA) represents one such method (5, 6), but it remains unclear whether siRNA will work as therapeutic agents in humans. We sought to develop a different approach, taking advantage of the 10-aa phosphopeptide sequence of IxB\(\alpha\) described above to target proteins for ubiquitination and degradation (4).

As proof of concept, we previously synthesized a chimeric...
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EXPERIMENTAL PROCEDURES

Synthesis of Protacs

IκBα Phosphopeptide-Estradiol Protac—To generate GA-1-mono-succinimidyl suberate, the estradiol derivative, GA-1 (7 mg, 11.5 μmol), was dissolved in 1 ml of anhydrous dimethylformamide (DMF), and disuccinimidyl suberate (21 mg, 57.0 μmol) was added at room temperature. After overnight stirring, DMF was removed under high vacuum, and the resulting white solid was flash-chromatographed to give GA-1-mono-succinimidyl suberate (6.3 mg, 7.3 μmol, 63.5%). For synthesis of GA-1-IκBα phosphopeptide, GA-1-mono-succinimidyl suberate (6 mg, 6.9 μmol) in DMDSO (1 ml) was added to dimethylsulfoxide (DMSO) solution (0.4 ml) containing IκBα phosphopeptide (1.5 mg, 0.92 μmol) and dimethylaminopropylidine (0.5 mg). After 30 min stirring at room temperature, the coupling reaction was completed, which was confirmed by a Kaiser test. DMDSO was removed under high vacuum, and the resulting crude product was repeatedly washed with dichloromethane and methanol to remove excess GA-1-mono-succinimidyl suberate to give the final product, GA-1-IκBα phosphopeptide (1.5 mg, 0.63 μmol, 68.5%). The final product was characterized by electrospray (ES) mass spectrometry. ES-MS (M+H)+ for GA-1-IκBα phosphopeptide was 2384.0 Da. All other intermediates were characterized by 500-MHz 1H nuclear magnetic resonance spectroscopy.

IκBα-DHT Protac—For DHT-Gly-mono-succinimidyl suberate, DMF (28 μl, 0.33 mmol) was added to dichloromethane solution (20 ml) containing Fmoc-Gly-Gly-OH (1.06 g, 3.57 mmol) and oxaclil chloride (0.62 ml, 7.10 μmol) at 0°C. After 3 h of stirring at room temperature, dichloromethane was removed under nitrogen atmosphere. The resulting solid residue was redissolved in dichloromethane (8 ml) and was combined with 5a-dihydrotestosterone (0.18 g, 0.62 mmol) and dimethylaminopropylidine (0.58 g, 4.75 mmol) in dichloromethane (20 ml) at 0°C. The reaction mixture was stirred overnight at room temperature. After dichloromethane was removed under reduced pressure, the resulting residue was flash-chromatographed to provide DHT-Gly-Fmoc (0.21 g, 0.37 mmol, 60%). Next, DHT-Gly-Fmoc (0.12 g, 0.21 mmol) was treated with tetrabutylammonium fluoride (0.3 ml, 1 M in tetrahydrofuran) at room temperature for 20 min, and the DMF was removed under high vacuum. The resulting residue was flash-chromatographed to provide DHT-Gly-NH2 (white solid, 49 mg, 0.14 mmol, 67%). Next, disuccinimidyl suberate (0.27 g, 0.73 mmol) was added to DMF solution (1 ml) containing DHT-Gly-NH2 (49 mg, 0.14 mmol) at room temperature. After overnight stirring, DMF was removed under high vacuum, and the resulting crude product was flash-chromatographed to give DHT-Gly-mono-succinimidyl suberate (70 mg, 0.12 mmol, 86%). DHT-Gly-mono-succinimidyl suberate (5.5 mg, 9.16 μmol) in DMSO (0.6 ml) was added to DMSO solution (1 ml) containing IκBα phosphopeptide (4.5 mg, 2.75 μmol) and dimethylaminopropylidine (2.0 mg, 16.37 μmol). After 20 min of stirring at room temperature, the coupling reaction was completed, which was confirmed by a Kaiser test. DMF was removed under high vacuum, and the resulting crude product was repeatedly washed with dichloromethane and methanol to remove excess DHT-Gly-mono-succinimidyl suberate to give the final product, DHT-IκBα phosphopeptide (3.5 mg, 1.65 μmol, 60%). The final product was characterized by ES mass spectrometry. ES-MS (M+H)+ for fumagillol-Gly-suberate-HIF-1α octapeptide was 2120 Da. All other intermediates were characterized by 500-MHz 1H nuclear magnetic resonance spectroscopy.

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GFP positive at the time of harvesting.

Ubiquitination Assays with ER—293T cell pellets were lysed with 200 μl of lysis buffer (25 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 5 mM NaF, 0.05 mM EGTA, 1 mM phenylmethylsulfonyl fluoride). Pellets from cells transfected with vector, pFLAG-β-TRCP, or pFLAG-Cul-1 were vortexed for 10 s, then incubated on ice for 15 min. After centrifugation at 13,000 rpm in an Eppendorf microfuge for 5 min at 4 °C, the supernatant was added to 20 μl of FLAG M2 beads (Sigma), which were washed with lysis buffer three times before immunoprecipitation. Lysates were incubated with the beads on a rotator for 2 h at 4 °C, followed by one wash with buffer A (25 mM HEPES buffer, pH 7.4, 0.01% Triton X-100, 150 mM NaCl) and one wash with buffer B (the same buffer without the Triton X-100). Ubiquitination assay was performed by mixing rabbit E1 (0.2 μg) the E2, Ubch5a (0.8 μg from Boston Biochem, West Bridgewater, MA), ubiquitin (5 μg) or methyl ubiquitin (1.5 μg), Protac (10 μM final concentration unless otherwise specified), recombinant ER (260 ng; from Invitrogen, Carlsbad, CA), and ATP (1 mM final concentration) in total reaction volume of 5.0 μl, which was then added to 20 μl (packed volume) of washed FLAG-M2 beads (7). Reactions were incubated for 1 h at 30 °C in a thermomixer (Eppendorf, Westbury, NY) with intermittent mixing. SDS-PAGE loading buffer was added to terminate the reactions. Western blot analysis was performed by standard methods using polyclonal anti-ER antiserum (1:10,000 dilution).

Degradation Experiments with Purified Yeast 26S Proteasome—Ubiquitination assays were performed as described above. Purified 26S yeast proteasomes (40 μl of 0.5 mg/ml) were added to the ubiquitinated ER on beads and the reaction was supplemented with 6 μl of 1 mM ATP, 2 μl of 0.2 mM magnesium acetate, and ubiquitin aldehyde 5 μl final concentration as previously described (16, 17). The reaction was incubated for 10 min at 30 °C with occasional shaking in a thermomixer. For ubiquitination studies, purified yeast 26S preparations were preincubated 45 min at 30 °C with the proteasome inhibitor 10 mM phenylmethylsulfonyl fluoride (Sigma) with intermitten mixing. SDS-PAGE loading buffer was added to terminate the reactions. Western blot analysis was performed by standard methods using polyclonal anti-ER antiserum (1:1000 dilution).

Microinjection Experiments—293 cells were transfected with a plasmid that expresses GFP-AR (kindly provided by Charles Sawyer, Howard Hughes Medical Institute, University of California, Los Angeles, CA) as described above. Cells were selected with G418 (600 μg/ml) and cultured in modified essential medium with penicillin, streptomycin, and l-glutamine. Prior to experiments, cells were −60% confluent in 6-cm dishes. Protac diluted to 10 μM in KCl (200 mM) with rhodamine dextran (molecular mass 10,000 Da; 50 μg/ml) was injected into cells through a microcapillary needle using a pressurized injection system (Picospritzer II; General Valve Corporation, Fairfield, NJ). The injected volume was 0.2 pl, representing 5–10% of the cell volume. For proteasome inhibition experiments, cells were treated with 10 μM epoxomicin (Calbiochem, La Jolla, CA) for 4 h or coincubated with epoxomicin (10 μM) and Protac (10 μM) (18, 19). Photographs were taken following injection using a Nikon 35 mm camera (Nikon, Melville, NY). GFP and rhodamine fluorescence were visualized with a Zeiss fluorescent microscope (Zeiss, Oberkochen, Germany).

RESULTS

Protacs consisting of the minimal 10-aa peptide (phosphorylated on the underlined S residues), DRHDSGLDSM covalently linked to either estradiol (E2; Protac-2) or DHT (Protac-3), were synthesized (Fig. 1). We first performed in vitro ubiquitation assays with both Protacs, but focused our efforts on Protac-2 due to problems encountered with expression of recombinant AR. To determine whether Protac-2 promotes the ubiquitination of ER by SCFβ-TRCP in a concentration-dependent manner, we performed ubiquitination assays with increasing concentrations of Protac (Fig. 2A). ER was ubiquitinated starting at a concentration of 0.1–1 μM Protac-2, with maximal efficiency observed at 5–10 μM. At 500 μM, we no longer observed ubiquitination of ER by SCFβ-TRCP, which may be due to a “squeathing” phenomenon wherein the presence of excess Protac-2 inhibits competitively the formation of heteromeric ER-Protac-2-SCF complexes. Because 10 μM Protac-2 promoted efficient ubiquitination of ER, we continued to use this concentration for the remainder of our studies (except as noted below). It should be noted that we consistently observed Cul1-dependent ubiquitination of ER in the absence of Protac-2 (e.g. Fig. 2, A and B, lane 1). This may be due to the presence of an ER-specific SCF ubiquitin ligase in the Cul1 precipitates. Regardless, these Protac-independent conjugates were of low molecular mass and clearly distinguishable from the high molecular mass, methyl ubiquitinsensitive conjugates induced by Protac-2 (e.g. compare lanes 1, 3, and 4 of Fig. 2B).

To address the mechanism of action of Protac-2, we tested whether the IxBα phosphopeptide and estradiol individually can compete out Protac-2, and whether these ligands when added together as free compounds can mimic the action of Protac-2. A 10-fold excess of either IxBα phosphopeptide (Fig. 2D) or estradiol (Fig. 2E) in cells completely blocked the ubiquitination-promoting activity of 1 μM Protac-2. Moreover, when added together as separate compounds, estradiol, and IxBα phosphopeptide failed to reproduce the effect of Protac-2 (Fig. 2C).

These results are consistent with our hypothesis that Protac-2 acts as a bridging molecule in that the estradiol moiety associates with the ER while the other moiety, the IxBα phosphopeptide, recruits the ER to the SCFβ-TRCP.

We next tested the specificity of Protac-mediated ubiquitination. Ubiquitination assays with ER were performed in the presence of either Protac-2, Protac-3, or a Protac (Protac-4) that consisted of the Zap70 phosphopeptide, which is recognized by the cbl ubiquitin ligase (20) and ovalicin, which binds MetAP-2 (8). As shown in Fig. 2F, ER was not ubiquitinated by SCFβ-TRCP in the presence of either Protac-3 or Protac-4.

Not all ubiquitin-ubiquitin linkages are able to sustain targeting to the proteasome (21), and possibly as a consequence substrates ubiquitinated under the relatively artificial conditions encountered in reconstituted systems can be poor substrates for the proteasome (22). Thus, we sought to determine whether ER-ubiquitin conjugates induced by Protac-2 were recognized by the 26S proteasome. To answer this question, purified yeast 26S proteasome (16) was added to ubiquitinated ER formed in the presence of SCFβ-TRCP and Protac-2. Complete disappearance of high molecular mass ubiquitin conjugates was observed within 10 min (Fig. 3A) and was partially blocked by the metal chelator 1,10 phenanthroline (which inhibits the essential Rpn11 isopeptidase activity of the proteasome), but not by the inactive derivative 1,7 phenanthroline (17) (Fig. 3B).
Our results with the IxBα phosphopeptide-estradiol Protac demonstrated that a medically relevant target protein can be recruited to a ubiquitin ligase through noncovalent interactions and be ubiquitinated and degraded in vitro. We next wished to test whether a Protac could promote the degradation of proteins in cells. For these experiments we used Pro-
Fig. 2. Protac-2 activates ubiquitination of ER in vitro. A, dose-dependent stimulation of ER ubiquitination by Protac-2. Purified ER was incubated with recombinant E1, E2, ATP, ubiquitin, and immobilized SCF\(^{β-TRCP}\) isolated from animal cells by virtue of FLAG tags on cotransfected Cul1 and β-TRCP. Reactions were supplemented with the indicated concentration of Protac-2, incubated for 60 min at 30 °C.
We further verified that the linkage of phosphopeptide and DHT was required for GFP-AR degradation. Coinjection of free I\textsubscript{xB\alpha} phosphopeptide and testosterone (10 \textmu M each) into 293 cells did not result in decreased GFP signal (Fig. 4C), indicating that intact Protac is necessary to promote degradation of GFP-AR. To determine whether GFP-AR degradation was dependent on I\textsubscript{xB\alpha} phosphopeptide and testosterone binding to their respective targets, we coinjected Protac-3 (10 \textmu M) with a 10-fold molar excess (100 \textmu M) of free phosphopeptide (Fig. 4D) or testosterone (Fig. 4E) into 293AR-GFP cells. In both cases, degradation of GFP-AR was inhibited. All experiments were performed on three separate days with 20–30 cells injected per experiment. The results shown are representative of the phenotype in greater than 70% of cells counted. Taken together, these data support the hypothesis that Protac-3 induced AR-GFP degradation by targeting AR-GFP to SCF\textsuperscript{\beta-TRCP}.

To determine whether the disappearance of GFP-AR was proteasome dependent, 293\textsuperscript{AR-GFP} cells were treated with the proteasome inhibitor epoxomicin for 4 h prior to injection with Protac-3 (10 \textmu M) (Fig. 4F). In cells treated with epoxomicin, GFP-AR was not degraded, suggesting that the Protac mediates degradation through a proteasome-dependent pathway. Cells were also coinjected with Protac (10 \textmu M) and epoxomicin (10 \textmu M) in the absence of pretreatment resulting in inhibition of GFP-AR degradation (data not shown). The result shown is representative of experiments performed on 3 different days with at least 30 cells injected per day.

As demonstrated previously (Fig. 2F), the I\textsubscript{xB\alpha} phosphopeptide-estradiol Protac-2, but not Protac-3, specifically induces ubiquitination of ER \textit{in vitro}. The specificity is dependent on the ability of Protac-2 to be recognized by the ubiquitin ligase as well as its ability to bind to ER. The same specificity of Protac action appears to hold true in cells, because Protac-2, unlike Protac-3, does not induce degradation of GFP-AR (Fig. 4G).

**DISCUSSION**

The ubiquitin-proteasome pathway rapidly, efficiently, and selectively ubiquitinates and degrades targeted polypeptides. Many signaling processes critical to the biology of normal and diseased cells are regulated by ubiquitin-dependent proteolysis, including exit from M phase of the cell cycle and initiation of innate immune response, which are respectively controlled by degradation of cyclin B and the NF-\textsuperscript{xB regulator} I\textsubscript{xB\alpha} (23, 24). To harness the power of the ubiquitin-proteasome pathway for therapeutic purposes, we are developing Protacs to

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**Fig. 3. Ubiquitinated ER is degraded by the 26S proteasome.** A, Ubiquitination reactions performed as described in the legend to Fig. 2A were supplemented with purified yeast 26S proteasomes. Within 10 min, complete degradation of ER was observed. B, Purified 26S proteasome preparations were preincubated in 1,10 phenanthroline (1 mM) or 1,7 phenanthroline (1 mM) prior to addition. The metal chelator 1,10 phenanthroline inhibits the Rpn11-associated deubiquitinating activity that is required for substrate degradation by the proteasome. Degradation of ER was inhibited by addition of 1,10 phenanthroline, but not the inactive derivative 1,7 phenanthroline.

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and monitored by SDS-PAGE followed by immunoblotting with an anti-ER antibody. B, Protac-2 induces assembly of high molecular weight mult ubiquitin chains on ER. Same as A, except that methyl ubiquitin was added in the place of ubiquitin (lane 4). C, estradiol and I\textsubscript{xB\alpha} phosphopeptide must be covalently linked to promote ER ubiquitination. The reaction was as described in A, except that I\textsubscript{xB\alpha} phosphopeptide and estradiol (5 \textmu M) were separately added to the ubiquitination reaction instead of Protac-2. D and E, free I\textsubscript{xB\alpha} phosphopeptide (D) and estradiol (E) compete out Protac activity. Same as A, except that Protac-2 was used at 1 \mu M. Increasing amounts of I\textsubscript{xB\alpha} phosphopeptide (lanes 2–5) or 10 \mu M of I\textsubscript{xB\alpha} peptide that is unphosphorylated (lane 6, arrow) was added to ubiquitination reaction. F, Protacs are target specific. Same as A, except that ZAP70-ovalcin and I\textsubscript{xB\alpha} phosphopeptide-DHT Protacs were used in place of Protac-2, as indicated.
recruit proteins to ubiquitin ligases to promote their ubiquitination and degradation. An important aspect of the Protacs approach is that it in theory can be applied to any protein in the cytoplasm or nucleus of a diseased cell, and thus may enable the development of therapeutics against a large fraction of proteins in the proteome. The linchpin of our approach is a heterobifunctional small molecule (i.e., Protac) that serves as a bridge to link a target protein to a ubiquitin ligase. Previously, we demonstrated that a Protac comprising a phosphopeptide that binds SCFβ-TRCP and a small molecule...
(ovalicin) that binds MetAP-2 activates the ubiquitination of MetAP-2 by SCF<sup>β-TRCP</sup> ubiquitin ligase in vitro, and consequently targets MetAP-2 for degradation by the proteasome in frog extract (7).

Our goals in the current work were to show that Protacs can increase the turnover of a given target protein in cells, and to extend the Protac approach to proteins that play a causal role in human diseases. We chose the estrogen and androgen receptors for our current studies due to their well-characterized association with estrogen and androgen, respectively. Furthermore, both receptors have been associated with the development and progression of cancer.

The results reported here indicate that Protacs operate by a bridging mechanism to enable efficient and specific down-regulation of ER in vitro and AR in cells. From our in vitro data, it is apparent that Protacs can be developed against different targets (MetAP-2, ER, and AR), and that Protacs promote ubiquitination of these targets in a manner that is both target selective and dose dependent.

From microinjection experiments, it is clear that Protacs can activate AR turnover in the context of the cellular degradation machinery. This degradation was also found to be specific and dependent on both components of the Protac molecule. Moreover, the proteasome inhibitor epoxomicin blocked the ability of Protacs to promote AR turnover, suggesting that the degradation is proteasome specific and not due to alternative pathways, such as those involving lysosomes, or due to other proteasomes, such as caspasas.

To deliver Protacs to cells in the experiments described here, we employed microinjection due to the impermeability of the SCF<sup>β-TRCP</sup>-binding LxBα phosphopeptide moiety. A key remaining challenge for Protac technology is to develop cell permeable molecules that can be used to test for efficacy in cell and animal models of cancer. Ongoing work in our laboratories suggests that Protacs based on the hydroxyproline motif of HIF1-α may be used to target ubiquitination and degradation of proteins in cells through the von-Hippel-Lindau ubiquitin ligase pathway.2

We postulate that many Protac compounds can be generated to treat a variety of diseases. First of all, hundreds of putative ubiquitin ligases that can be exploited as agents of Protac action have been uncovered by the Human Genome Project. Second, it is important to note that Protacs should not be limited to receptors with well-defined ligands such as AR and ER. In theory, any protein that binds a small molecule can activate the ubiquitination of these targets in a manner that is both target selective and dose dependent.

Acknowledgments—We thank Charles Sawyers for providing the GFP-AR expression plasmid and Eric Davidson (Division of Biology, Caltech, La Jolla, CA) for use of the microinjection equipment and microscope. We are also grateful to Frank Mercurio (Signal Division, Celgene Pharmaceuticals, Warren, NJ) for help obtaining GA-1-monomosuccinimidyl suberate.

This work was supported by the University of California Los Angeles SPORE in Prostate Cancer Development Research Seed Grant (P50 CA92131 to K. M. S.), CaPCURE (to R. J. D., C. M. C., and K. M. S.), Department of Defense (DAMD17-03-1-0220 to K. M. S.), UC BioSTAR Project (01-10232 to K. M. S.), Stein-Oppenheimer Award (K. M. S.), and the Susan G. Komen Breast Cancer Foundation (DISS021703 to R. J. D.). R. J. D. is an Assistant Investigator of the HHMI. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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REFERENCES

1. Debes, J. D., Schmidt, L. J., Huang, H., and Tindall, D. J. (2002) P300 coactivator interacts with SKP2 to promote ubiquitination and degradation of hyperphosphorylated p53. <em>Mol. Cell</em> *10,* 435–437

6. Tuschi, T. (2002) Expanding small RNA interference. <em>Nat. Biotechnol.</em> *20,* 446–448

7. Sakamoto, K. M., Kim, K. B., Kumatagi, A., Mercurio, F., Crews, C. M., and Deshaies, R. J. (2001) Protacs: Chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. <em>Proc. Natl. Acad. Sci. U. S. A.</em> 98, 8554–8559

8. Sin, N., Meng, L., Wang, M. Q., Wen, J. J., Bommann, W. G., and Crews, C. M. (1997) The anti-angiogenic agent tamagulin covalently binds and inhibits the methionine aminopeptidase, MetAP-2. <em>Proc. Natl. Acad. Sci. U. S. A.</em> 94, 6099–6103

9. Howell, A., Howell, S. J., and Evans, D. G. (2003) New approaches to the endocrine prevention and treatment of breast cancer. <em>Cancer Chemother. Pharmacol.</em> 52 (Suppl 1), 39–44

10. Debes, J. D., Schmidt, L. J., Huang, H., and Tindall, D. J. (2002) P300 mediates androgen-independent transactivation of the androgen receptor by interleukin 6. <em>Cancer Res.</em> 62, 5632–5636

11. Levenson, A. S., Svoboda, K. M., Pease, K. M., Kaiser, S. A., Chen, B.,...
Simons, L. A., Jovanovic, B. D., Dyck, P. A., and Jordan, V. C. (2002) Gene expression profiles with activation of the estrogen receptor alpha-selective estrogen receptor modulator complex in breast cancer cells expressing wild-type estrogen receptor. Cancer Res. 62, 4419–26

12. Cardozo, C. P., Michaud, C., Ost, M. C., Fliss, A. E., Yang, E., Patterson, C., Hall, S. J., and Caplan, A. J. (2003) C-terminal Hsp-interacting protein slows androgen receptor synthesis and reduces its rate of degradation. Arch. Biochem. Biophys. 410, 134–40

13. Lonard, D. M., Nawaz, Z., Smith, C. L., and O'Malley, B. W. (2000) The 26S proteasome is required for estrogen receptor-alpha and coactivator turn-over and for efficient estrogen receptor-alpha transactivation. Mol. Cell. 5, 939–48

14. Nawaz, Z., Lonard, D. M., Dennis, A. P., Smith, C. L., and O’Malley, B. W. (1999) Proteasome-dependent degradation of the human estrogen receptor. Proc. Natl. Acad. Sci. U. S. A. 96, 1858–62

15. Zhou, P., Bogacki, R., McReynolds, L., and Howley, P. M. (2000) Harnessing the ubiquitination machinery to target the degradation of specific cellular proteins. Mol. Cell 6, 751–756

16. Verma, R., Chen, S., Feldman, R., Schieltz, D., Yates, J., Dohmen, J., and Deshaies, R. J. (2000) Proteasomal proteomics: Identification of nucleotide-sensitive proteasome-interacting proteins by mass spectrometric analysis of affinity-purified proteasomes. Mol. Biol. Cell 11, 3425–3439

17. Verma, R., Aravind, L., Oania, R., McDonald, W. H., Yates, J. R., 3rd, Koonin, E. V., and Deshaies, R. J. (2002) Role of Rpn11 metalloprotease in deubiquitination and degradation by the 26S proteasome. Science 298, 611–615

18. Myung, J., Kim, K. B., Lindsten, K., Dantuma, N. P., and Crews, C. M. (2001) Lack of proteasome active site allosteric as revealed by subunit-specific inhibitors. Mol. Cell 7, 411–420

19. Dantuma, N. P., Lindsten, K., Glas, R., Jelline, M., and Masucci, M. G. (2000) Short-lived green fluorescent proteins for quantifying ubiquitin/proteasome-dependent proteolysis in living cells. Nat. Biotechnol. 18, 538–543

20. Zheng, N., Wang, P., Jeffrey, P. D., and Pavletich, N. P. (2000) Structure of a c-Cbl-UbcH7 complex: RING domain function in ubiquitin-protein ligases. Cell 102, 533–539

21. Pickart, C. M. (2000) Ubiquitin in chains. Trends Biochem. Sci. 25, 544–548

22. Petroski, M. D., and Deshaies, R. J. (2003) Context of mult ubiquitin chain attachment influences the rate of Sic1 degradation. Mol. Cell 11, 1435–1444

23. Karin, M., and Ben-Neriah, Y. (2000) Phosphorylation meets ubiquitination: The control of NF-κB activity. Annu. Rev. Immunol. 18, 621–663

24. Zachariae, W., and Nasmyth, K. (1999) Whose end is destruction: Cell division and the anaphase-promoting complex. Genes Dev. 13, 2039–2058

25. Druker, B. J., Sawyers, C. L., Kantarjian, H., Resta, D. J., Reese, S. F., Ford, J. M., Capdeville, R., and Talpaz, M. (2001) Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N. Engl. J. Med. 344, 1038–1042

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