When protein destruction runs amok, malignancy is on the loose

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Ubiquitin-dependent proteolysis ensures that specific protein functions are turned off at the right time, in the right place, and in a unidirectional fashion. The high substrate specificity of the system is determined by a large family of ubiquitin ligases, which competes with the protein kinases to be the largest family of enzymes in mammals. Given the crucial function of the proteolytic machinery, altered degradation of cellular regulators contributes to the unchecked proliferation typical of cancer cells. Here we review the aberrant activity of a variety of ubiquitin ligases in human cancer, hence the prospect of targeting them in cancer therapy.

There is a plethora of evidence showing that in order to keep a cell healthy, proteins need to be destroyed in a timely fashion. Regulated protein degradation offers the advantage of switching off a function in a specific subcellular compartment while leaving the others untouched, a task that the cell could not perform if protein abundance was regulated only by synthesis. In addition, proteolysis has the advantage of being fast, as a large number of different proteins can be eliminated in a matter of minutes. Last, but not least, proteolysis is irreversible, an essential quality to temporally limit a cellular function (e.g., that of a transcription factor) or permanently eliminate a protein in unidirectional processes such as the cell division cycle. Cells deploy the ubiquitin-proteasome system to degrade cellular regulatory proteins, thus controlling their abundance. The targeting of a substrate to the 26S proteasome requires the covalent attachment of polyubiquitin chains to one or multiple lysine residues of the substrate. Upon association with the proteasome, the ubiquitin molecules are recycled and the substrate fed into the catalytic core where it is proteolyzed (Hershko and Ciechanover, 1998; Pickart, 2001). Protein ubiquitinylation is a multistep process orchestrated by the concerted action of three enzymes. The chain reaction begins with a ubiquitin-activating enzyme (E1) forming a thiolester bond with the ubiquitin (a 76 amino acid peptide) in an ATP-dependent reaction. This is followed by a *ubi*quitin-*c*onjugating enzyme (Ubc or E2) that receives the activated ubiquitin from E1 to subsequently attach multiple ubiquitin moieties to the target protein. Finally, a ubiquitin-protein ligase (E3) recruits the target, guides the transfer of the ubiquitin from the Ubc to the substrate, and allows for elongation of the ubiquitin chain. Given the diversity of the target proteins, there is a corresponding large number of E3s that are classified into three groups. The first group is composed of single subunit RING finger-based E3s (e.g., Cbl, Mdm2), a family with more than 500 members in mammals, all showing residues within the RING motif that bind two atoms of Zinc. The second group, which includes several hundred members, is characterized by multisubunit RING finger protein complexes (e.g., SCF ubiquitin ligases [*S*kp1/*C*ul1/*F* box protein/Roc1] and the VBC ubiquitin ligase [*V*hl/Cul2/Elongin *B*/Elongin *C*/Roc1]). Finally, a third group of 61 members in mammals, the HECT-based E3s (e.g., Smurf, Nedd4), is characterized by a *h*omology to *E*6ap (the prototype of this family) *c*arboxy-*t*erminal domain. HECT E3s, in contrast to all the other ubiquitin ligases, are able to form a thiolester bond with the ubiquitin and directly transfer it to their substrates.

Several E3 enzymes have been shown to play a critical role in regulating cell proliferation, differentiation, or apoptosis. Because of this, the ubiquitin system is often the target of cancer-related deregulation and is involved in processes such as oncogenic transformation and tumor progression. Indeed, increased stability of positive regulators of proliferation (often protooncoproteins, such as cyclins, Notch, β-catenin, and c-Myc) can be achieved by lowering the activity or the levels of the specific enzymes necessary for their degradation. Thus, the ubiquitinylating enzymes specific for protooncoproteins can act as tumor suppressors. Accordingly, other ubiquitinylating enzymes could be oncogenic if their specific function is to target tumor suppressors (e.g., p53, pRb, p27). There are many established examples, some of which are summarized below, of both inactivation and overactivation of the ubiquitin pathway (especially of ubiquitin ligases) in human tumors.

Inactivation of the ubiquitin system in human cancers

The best-established example of inactivation of the ubiquitin system in the development of cancer is represented by the germline mutations in the tumor suppressor *VHL* (*v*on *H*ippel *L*indau) gene. These mutations are causative agents for the VHL syndrome, an autosomal dominant familial cancer syndrome predisposing individuals to tumors including renal cell carcinomas, cerebellar hemangioblastomas, hemangiomas, retinal angiomas, and pheochromocytomas (Conaway and Conaway, 2002). Significantly, spontaneous renal clear-cell carcinomas often also display somatic deletion of the *VHL* gene. Vhl is the substrate receptor of a Cul2-dependent VBC ubiquitin ligase targeting the *h*ypoxia *i*nducible transcription *f*actor subunits Hif1α and Hif2α under normoxic conditions (Figure 1). The HIF complex transcriptionally activates the expression of a variety of genes that induce angiogenesis. In normal cells, hypoxic stress inhibits the interaction between Vhl and Hifα subunits resulting in the accumulation of the latter with subsequent tissue vascularization (Kaelin, 2002). In individuals affected by VHL syndrome, somatic inactivation of the wild-type allele by deletions, mutations, or hypermethylation induces tumor development. Although Hif1α contributes to the hypervascularization typical of tumors present in the VHL syndrome, Hif2α appears to be the major oncogenic substrate of Vhl (Seagroves and Johnson, 2002).

Another case in which the substrate-targeting subunit of a cullin-dependent ligase is found mutated in cancer is that of the

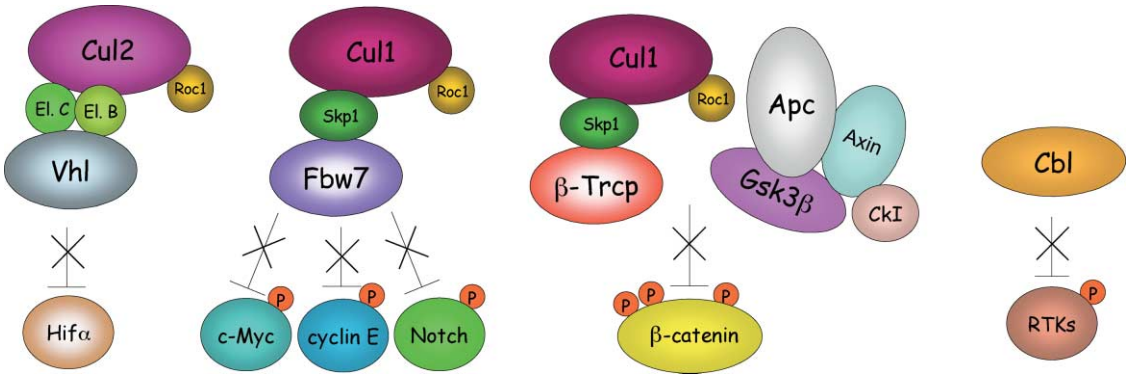

Figure 1. Inactivation of the ubiquitin system and cancer

Mutations in the genes encoding certain substrate-targeting subunits of cullin-dependent ubiquitin ligases (e.g., Vhl and Fbw7), facilitators of substrate-E3 interactions (e.g., Apc), or single subunit RING finger E3s (e.g., Cbl) all provide a gain of function thereby contributing to cellular transformation. In all but one example shown in this figure, these mutations result in the stabilization of positive regulators of cell proliferation (c-Myc, cyclin E, Notch, β-catenin). In the remaining one, loss of *VHL* facilitates angiogenesis under normoxic conditions providing a growth advantage to the tumor. See text for details. A circled "P" in red indicates phosphorylation of the substrate, which is necessary for recognition by the ubiquitin ligase.

F box protein Fbw7 (*F b*ox and *W*D-40 domain containing protein 7, also called human Sel10 and hCdc4), which targets cyclin E for ubiquitinylation. There is evidence that overexpression of cyclin E and that of cyclin D1 (another G1 cyclin) contribute to the development of several types of neoplasms and that their levels directly correlate with poor survival. Overexpression of cyclin D1 appears often to be the consequence of gene amplification, whereas the mechanism by which tumor cells increase cyclin E levels is less clear. In some human carcinomas and tumor epithelial cell lines, high levels of cyclin E have been attributed to its stabilization. It has been shown that *FBXW7*, encoding Fbw7, is mutated in human breast and ovary cells lines (Bartek and Lukas, 2001) and in approximately 16% of human endometrial tumors (Spruck et al., 2002), rendering it liable for the stabilization of cyclin E. Additional Fbw7 substrates such as c-Myc (B. Clurman, I. Hariharan, and K. Nakayama, personal communication), Notch1, and Notch4 (Lai, 2002) are very likely to contribute to transformation promoted by Fbw7 mutations (Figure 1).

β-Trcp (β-*t*ransducin *r*epeat *c*ontaining *p*rotein) represents a second example of an F box protein that during oncogenesis becomes unable to target one of its substrates, the protooncoprotein β-catenin. However, in this case, the defect is not intrinsic to the ubiquitin ligase but to the apparatus necessary for β-catenin phosphorylation and consequent recognition by β-Trcp. A protein complex including Apc (*a*denomatous *p*olyposis *c*oli), Axin/Conductin, Gsk3β (*G*lycogen *s*ynthase *k*inase-3β), and CkI (*C*asein *k*inase I) is required for the phosphorylation of cytoplasmic β-catenin (Polakis, 2002). CkI creates a priming site for Gsk3β, which in turn phosphorylates β-catenin on its destruction motif and allows it to be recognized by β-Trcp. A region of the *APC* tumor suppressor gene encodes a group of highly conserved amino acids involved in the binding and degradation of β-catenin. Mutations in this region or loss of the *APC* gene cause limited and inadequate phosphorylation of β-catenin resulting in its stabilization and nuclear translocation followed by transcriptional activation of proliferative-associated genes (Polakis, 1999) (Figure 1). A cellular mishap due to a mutation of the *APC* gene can be one of the leading factors in initiating the neoplastic process in normal colonic epithelial cells that succumb to the adenoma-carcinoma sequence of events (Lynch and de la Chapelle, 2003).

Inactivation of single subunit RING finger-based E3s, such as Cbl, is also associated with oncogenesis. Cbl recognizes phosphotyrosine residues on *r*eceptor *t*yrosine *k*inases (RTKs) and plays a central role in negatively regulating signaling by promoting receptor ubiquitinylation (Peschard and Park, 2003). Transforming variants of the Cbl protooncoprotein containing deletions or point mutations in the C-terminal RTK binding domain are found to have lost the ability to ubiquitinylate RTKs (Figure 1). This defect leads to ligand-independent activation or enhanced catalytic activity of RTKs, both of which can be contributing factors to cell transformation. Loss of the ubiquitin ligase activity, however, participates but is not on its own sufficient to induce transformation since some mutations that abolish the ligase activity are not transforming.

Another RING finger E3, Brca1, which is the product of a tumor suppressor gene (*BR*east *CA*ncer susceptibility gene *1*), has been linked to cancer development. Although originally identified as a single subunit RING finger-based E3, it is now clear that Brca1 acts mostly in a heterodimeric complex with another RING finger protein named Bard1 (*B*rca1-*a*ssociated *R*ING *d*omain 1). Germline mutations in one *BRCA1* allele predispose women to early onset familial breast and ovarian cancers due to the inactivation of the remaining wild-type allele by somatic mutations (Baer and Ludwig, 2002). Many tumor-associated mutations in *BRCA1* are localized in the RING motif, while others are thought to affect the substrate binding region. Similarly, mutations in the *BARD1* gene have been identified in breast, ovarian, and endometrial cancers. However, substrates of the Brca1/Bard1 complex have not been identified and, thus, it is not clear which one(s) stabilization contributes to transfor-

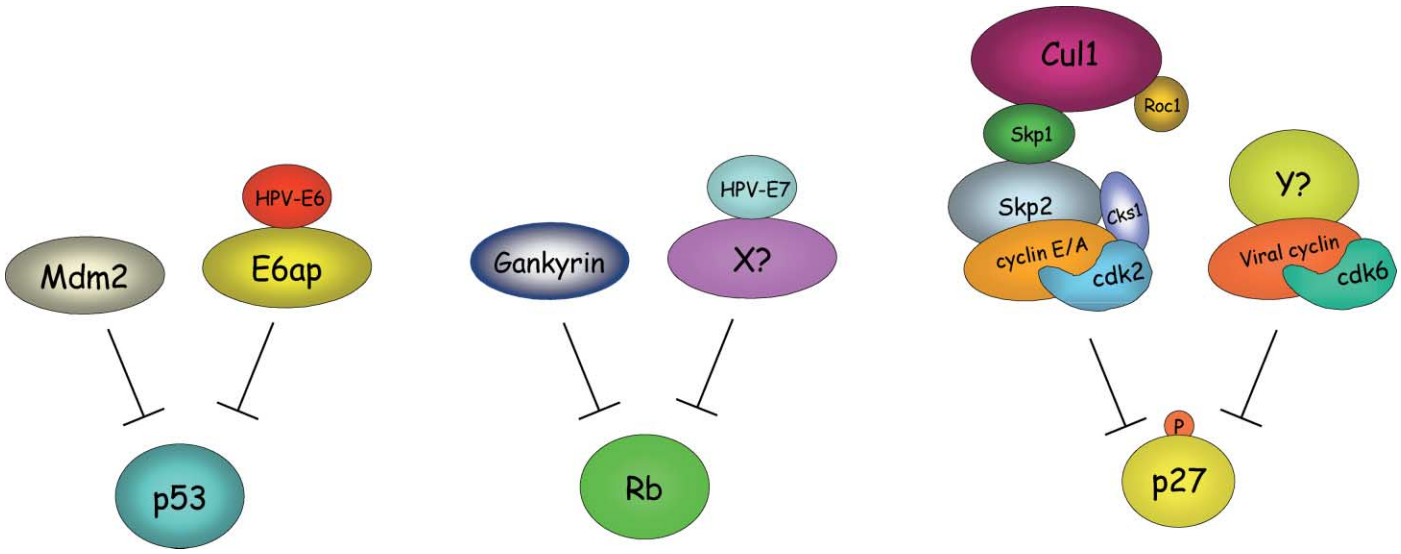

Figure 2. Overactivation of the ubiquitin system and cancer

Overexpression of Mdm2, Gankyrin, and Skp2 has been linked to various cancer types, suggesting that they are the products of protooncogenes. They all result in the enhanced degradation of tumor suppressors (p53, Rb, and p27, respectively). Oncogenic viruses (e.g., HPV and Kaposi's virus) also developed various strategies to eliminate tumor suppressor proteins using the cellular proteolytic machinery. See text for details.

mation in the absence of Brca1. Since it has been recently shown that FancD2, a protein implicated in the process of homologous recombination-mediated DNA repair, can be ubiquitinylated even in the absence of the Brca1/Bard1 complex (Vandenberg et al., 2003), the only other proposed candidate to be a Brca1/Bard1 substrate remains the large subunit of the RNA polymerase II, which is degraded in response to DNA damage (Baer and Ludwig, 2002). It is anticipated that loss of ubiquitinylation of substrates downstream to the Brca1/Bard1 complex deregulates DNA repair checkpoints.

Overactivation of the ubiquitin system in human cancers

The most well-established example of this kind is that of Mdm2, the product of a protooncogene originally identified as the *m*urine *d*ouble *m*inute 2 gene product based on its amplification in a spontaneously transformed derivative of mouse BALB/cells (Chene, 2003). The amplification of the *MDM2* locus has been observed in human sarcomas as well as in a variety of other tumor types, and often it translates in an overexpression of Mdm2 (Michael and Oren, 2002). Mdm2 is a negative regulator of the tumor suppressor p53, a critical component of cell cycle checkpoints in mammalian cells (Hainaut and Hollstein, 2000) (Figure 2). Physical interaction of Mdm2 with p53 silences the transcriptional activity of the latter and at the same time induces its ubiquitinylation and consequent degradation. Interestingly, in human tumors, amplification of *MDM2* and mutations in *TP53* (the gene encoding p53) are mutually exclusive due to their redundancy. Thus, tumor cells can eliminate p53 either via its mutation or by enhancing its degradation due to an increase in Mdm2 expression.

The proteolysis of other key tumor suppressors, such as Rb and p27, can be enhanced in human tumors (Figure 2). Gankyrin, a cellular oncoprotein overexpressed in hepatocellular carcinomas, physically interacts with Rb and counteracts its activity both by increasing its phosphorylation (probably by facilitating the interaction between Rb and Cdk4) and by destabilizing it (Dawson et al., 2002; Higashitsuji et al., 2000). However, how Gankyrin promotes proteasomal degradation of Rb has yet to be elucidated. As for the cdk inhibitor p27, its increased proteolysis, which results in its low levels of expression, has been described in aggressive lymphomas and human carcinomas, such as colon, breast, prostate, and lung cancers (Bloom and Pagano, 2003). Significantly, p27 destabilization correlates with poor prognosis and progression of the disease (Philipp-Staheli et al., 2001; Slingerland and Pagano, 2000). Increased degradation of p27 observed in human tumors correlates with an increase in the levels of the F box protein Skp2 that promotes the ubiquitinylation of p27 by targeting it to an SCF ligase. It has been shown that in some cancers, an amplification of the *SKP2* locus correlates with an overexpression of the protein (Dowen et al., 2003; Yokoi et al., 2002). Due to its role in downregulating p27, *SKP2* is to be considered as a protooncogene. Indeed, experiments in cultured cells and transgenic mice confirmed this hypothesis (Bloom and Pagano, 2003). As in the case of Skp2, the expression of Cks1, a cofactor of Skp2, may also be elevated in certain human cancers (D. Hershko, M. Loda, and M.P., unpublished results), suggesting that Skp2 and Cks1 act in synergy to downregulate p27 in cancer. Recent data suggest that Skp2 overexpression may also contribute to transformation by activating c-Myc, although the exact molecular mechanism of this process is still elusive (Jin and Harper, 2003).

Finally, other subunits of ubiquitin ligases have been found overexpressed in human tumors. For example, *CUL4a*, a gene involved in the regulation of DNA repair and DNA replication, is amplified in 16% of primary breast cancers (Chen et al., 1998). Levels of Emi1 (*e*arly *m*itotic *i*nhibitor 1), an F box protein that inhibits the activity of the APC/C (*a*naphase-*p*romoting *c*omplex/*c*yclosome) ubiquitin ligase, are often upregulated in many human carinomas, causing an accumulation of APC/C sub-

strates such as cyclins, Aurora-A, and Securin, hence predisposing to fault mitosis and genomic instability (Peters, 2003).

Cellular tumor suppressors, such as p27, Rb, and p53 are also destabilized by oncogenic DNA viruses that have developed strategies to get rid of them by inducing their proteolysis (Figure 2). Degradation of p27 is enhanced by the *h*uman *h*erpes*v*irus 8 (HHV8), which appears to be a causative agent for Kaposi's sarcoma and primary effusion lymphoma (Ellis et al., 1999; Mann et al., 1999). HHV8 encodes a protein with homology to cellular cyclins, called K cyclin (also known as viral cyclin or V cyclin), capable of circumventing a G1 arrest imposed by p27 and giving way to entry into S phase regardless of the presence of this cdk inhibitor. It accomplishes this feat by forming a complex with Cdk6 and upon phosphorylation of p27 promotes downregulation of p27 by the ubiquitin pathway, getting past G1 arrest. Since SCF^{Skp2} can target p27 only when this is bound to cyclin E/A-cdk2 complexes (Bloom and Pagano, 2003), it is not clear whether K cyclin-Cdk6, by phosphorylating and binding to p27, recruits it to SCF^{Skp2} or to a different ligase. *H*uman *p*apilloma*v*iruses (HPV) 16 and 18, two viruses implicated in the pathogenesis of human anogenital cancer, encode two major transforming proteins, E6 and E7. HPV E7 oncoprotein can efficiently eliminate Rb utilizing two routes: it is capable of binding to and sequestering Rb and at the same time it induces its proteasomal degradation via a yet to be discovered mechanism (Munger et al., 2001). HPV E6 acts upon another tumor suppressor since it functions as a bridge between the cellular ubiquitin ligase E6ap and p53, thus targeting the latter for degradation (Brown and Pagano, 1997). Similarly, the adenoviral proteins E1b55 and E4orf6 function together in a complex to promote p53 turnover by targeting to it a Cul5-dependent ubiquitin ligase (Cul5/Elongin B/Elongin C/Roc1) (Querido et al., 2001).

Stabilizing mutations are selected in oncoproteins

Stabilization of β-catenin, c-Myc, RTKs, and cyclins can occur not only because of mutations in the genes necessary to degrade them, as above discussed, but also because they can themselves be subject to mutations that prevent their ubiquitinylation. For example, human colorectal cancers wild-type for the *APC* gene are often associated with hotspot mutations in the *CATNB* gene encoding β-catenin, mostly affecting phosphorylation sites essential for its interaction with β-Trcp and its consequent degradation (Polakis, 1999). Human Burkitt's lymphoma, AIDS-associated lymphomas, and certain acute lymphoblastic leukemias are associated with translocations involving *c-MYC* (Boxer and Dang, 2001). However, in certain lymphomas, c-Myc accumulates because of an increase in its half-life. Hotspot mutations, particularly those present in the Myc-box 1, result in stabilization of c-Myc due to inefficient Fbw7-mediated recognition and subsequent ubiquitinylation (Jin and Harper, 2003) (K. Nakayama, personal communication). Interestingly, there is evidence that c-Myc is also stabilized by the X protein of *H*epatitis *B* virus (HB) as it inhibits its phosphorylation (V. Kumar, personal communication). HB has been shown to target also the cell cycle machinery since its integration in the *CCNA2* gene in a hepatocellular carcinoma results in a truncated cyclin A protein (Wang et al., 1990, 1992). The N terminus of cyclin A, including the signal necessary for its degradation by the APC/C ubiquitin ligase, is replaced by viral PreS2/S sequences. This chimeric protein is able to activate Cdk1 and Cdk2 as the wild-type cyclin A does, but being much more stable, it promotes uncontrolled cell proliferation. There are a few additional examples of mutations of oncoproteins that allow them to bypass their destruction. Many RTK-derived oncoproteins (e.g., the colony-stimulating factor 1, the receptor for epidermal growth factor, and the hepatocyte growth factor receptor) avoid Cbl-mediated downregulation because of mutations on their Cbl binding sites (Peschard and Park, 2003). In certain leukemias, an increased stability is observed in mutants of c-Myb that have lost the degradation signal at the carboxyl terminus necessary for the proteasomal degradation of the wild-type protein (Bies and Wolff, 1997). Similarly, the viral oncogene v-Myb differs from its cellular counterpart for its truncation at the carboxyl terminus, which in turn increases its stability (Grasser et al., 1991). v-Jun, a viral oncogenic form of the transcription factor c-Jun, is able to escape proteasomal degradation in an analogous fashion due to loss of the δ domain at the amino terminus, which is a degradation motif in the wild-type protein (Treier et al., 1994). The ubiquitin ligases targeting c-Myb and c-Jun are not yet known; however, by analogy to the E3 for c-Myc, it is possible that they are products of tumor suppressor genes.

The ubiquitin-proteasome system as a therapeutic target in cancer

Several proteasome inhibitors have been discovered that readily penetrate cells and selectively inhibit the proteasome, either reversibly or irreversibly. This year the US Food and Drug Administration has approved bortezomib (formerly known as PS-341) as the first proteasome inhibitor to be used for cancer therapy, namely in the therapy of myeloma. In phase II trials with bortezomib, 35% of patients with refractory and relapsed multiple myeloma showed response to therapy with additional patients exhibiting stabilization of the disease (Richardson, 2003). In addition, 10% achieved complete responses as defined by a normal serum myeloma protein concentration on electrophoresis. At a molecular level, bortezomib binds reversibly to the chymotryptic active sites of the proteasome with high potency and specificity but, despite much speculation, the nature of the crucial targets whose degradation is inhibited by bortezomib in myeloma remains to date unknown (Hideshima and Anderson, 2002). While one might have naively imagined that a general inhibitor of the proteasome may have generated pleiotropic effects precluding its use as a therapeutic agent, the experience with bortezomib provides evidence in support of its clinical use.

The success with bortezomib raises a strong interest in identifying additional proteasome inhibitors that might have different tumor specificity. However, despite the manageable side effects of bortezomib, it has been shown that proteasome inhibition is achieved not only in tumor cells but at a higher degree even in normal tissues (Adams, 2003; LeBlanc et al., 2002; Luker et al., 2003). In addition, although bortezomib is considered a reversible inhibitor of the proteasome, long regimen of bortezomib in mice showed an enhanced proteasome inhibition that no longer returned to baseline (Luker et al., 2003). Thus, proteasome antagonists will be able to prolong the survival of certain tumor patients, but it might not be utilized in those cancer patients who are in need of chronic treatments. Based on these considerations and given the diversity of E3 substrates and the multiplicity of specific ubiquitin ligases, the future will reward the search for small molecules aimed at inhibiting or activating E3s in order to construct more selective approaches that take into account different ligase-substrate pairs according

to each specific case. For example, the therapy of patients with tumors expressing low levels of p27 would benefit from Skp2 inhibitors, whereas cancers expressing high levels of cyclin E and/or c-Myc could be fought by agonists of Fbw7 (in tumors in which Fbw7 is wild-type). Therapeutics destabilizing Hif1α and Hif2α (perhaps by activating Vhl) could be used as anti-angiogenic agents. On the basis of results with siRNA oligos (L. Busino, M.P., and G. Draetta, unpublished results) and dominant-negative mutants (Soldatenkov et al., 1999), inhibitors of β-Trcp are expected to sensitize tumor cells to DNA damage. Similarly, it is predicted that stabilization of p53 by inhibition of Mdm2 would induce apoptosis in cancers that express wild-type p53. Finally, since recent studies demonstrate that TGF-β signaling is required for promotion of invasive/metastatic behavior in several late-stage cancers (Roberts and Wakefield, 2003), compounds that stimulate the activity of Smurf, a HECT E3, would specifically promote degradation of the TGF-β receptor. This would result in the inhibition of TGF-β signaling and consequently provide a novel approach to prevent metastasis in late-stage disease.

Based on these perspectives, many biotech companies and large pharmaceutical industries are currently dedicated to screen for modulators of E3 ubiquitin ligases. The functions of E3s can be inhibited either by interfering with substrate interactions or by directly repressing the activity of the E3 itself. Inhibitors of Skp2 have been identified that upregulate p27 and induce apoptosis in certain cancer cell lines (W. Xie and M.P., unpublished results). Although approaches to promote the activity of an E3 are more difficult to envision, small molecules increasing the ubiquitin ligase activity of Smurf both in vitro and in cultured cells have been developed (F. Mercurio, personal communication). Other strategies can be used to modulate the stability of a substrate without interfering with its binding to its specific E3 or with the activity of the latter. For example, CI-1033, a compound being evaluated in phase I trials is a pan-ErbB receptor tyrosine kinase inhibitor but also induces the destabilization of ErbB receptors, probably by inducing their dimerization and consequent binding to their specific E3, namely Cbl (Citri et al., 2002). Another compound called CP-31398 is able to stabilize wild-type p53 and to restore a wild-type conformation in p53 mutants (Wang et al., 2003). Although the mechanism by which CP-31398 blocks p53 ubiquitinylation is not understood, a therapy utilizing a drug with such characteristics could be of benefit in tumors expressing wild-type p53 (and eventually high Mdm2 levels) as well as in cancers with p53 mutations.

In summary, as the examples mentioned above illustrate, timely degradation of proteins that control cell proliferation and apoptosis is vital in keeping normal growth from turning into runaway malignancy. In particular, E3 ubiquitin ligases play a critical role in cellular processes that are linked to tumorigenesis. Therefore they represent an important class of potential drug targets for anticancer therapy. It is noteworthy that there are additional enzymes of the ubiquitin-dependent protein destruction web that may prove of interest in human cancer treatment. For example, a large family of deubiquitinylating enzymes is present in mammals and, although their biological function remains almost completely elusive, it is possible that their activity might counteract that of ubiquitin ligases (Wilkinson, 2000). Connecting the increasing number of dots corresponding to the various E3s and their respective substrates and understanding the signals that regulate specific ubiquitin ligation events will brighten the future of targeting the ubiquitin system for anti-cancer therapies. While proteasome inhibition has proved to be of therapeutic utility, the strategy of modulating the activity of E3 ubiquitin ligases (and perhaps deubiquitinylating enzymes) in order to get rid of oncoproteins or increase the levels of tumor suppressors is definitely more specific and will present fewer side effects. Depending on the molecular characteristics of the different tumors, one could envisage targeting specific E3s in different cancers as a valid approach in future clinical oncology.

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References

Adams, J. (2003). The proteasome: structure, function, and role in the cell. Cancer Treat. Rev. *29* Suppl. 1, 3–9.

Baer, R., and Ludwig, T. (2002). The BRCA1/BARD1 heterodimer, a tumor suppressor complex with ubiquitin E3 ligase activity. Curr. Opin. Genet. Dev. *12*, 86–91.

Bartek, J., and Lukas, J. (2001). Cell cycle. Order from destruction. Science *294*, 66–67.

Bies, J., and Wolff, L. (1997). Oncogenic activation of c-Myb by carboxyl-terminal truncation leads to decreased proteolysis by the ubiquitin-26S proteasome pathway. Oncogene *14*, 203–212.

Bloom, J., and Pagano, M. (2003). Deregulated degradation of the cdk inhibitor p27 and malignant transformation. Semin. Cancer Biol. *13*, 41–47.

Boxer, L.M., and Dang, C.V. (2001). Translocations involving c-myc and c-myc function. Oncogene *20*, 5595–5610.

Brown, J.P., and Pagano, M. (1997). Mechanism of p53 degradation. Biochim. Biophys. Acta *1332*, O1–O6.

Chen, L.C., Manjeshwar, S., Lu, Y., Moore, D., Ljung, B.M., Kuo, W.L., Dairkee, S.H., Wernick, M., Collins, C., and Smith, H.S. (1998). The human homologue for the Caenorhabditis elegans cul-4 gene is amplified and overexpressed in primary breast cancers. Cancer Res. *58*, 3677–3683.

Chene, P. (2003). Inhibiting the p53-MDM2 interaction: an important target for cancer therapy. Nat. Rev. Cancer *3*, 102–109.

Citri, A., Alroy, I., Lavi, S., Rubin, C., Xu, W., Grammatikakis, N., Patterson, C., Neckers, L., Fry, D.W., and Yarden, Y. (2002). Drug-induced ubiquitylation and degradation of ErbB receptor tyrosine kinases: implications for cancer therapy. EMBO J. *21*, 2407–2417.

Conaway, R.C., and Conaway, J.W. (2002). The von Hippel-Lindau tumor suppressor complex and regulation of hypoxia-inducible transcription. Adv. Cancer Res. *85*, 1–12.

Dawson, S., Apcher, S., Mee, M., Higashitsuji, H., Baker, R., Uhle, S., Dubiel, W., Fujita, J., and Mayer, R.J. (2002). Gankyrin is an ankyrin-repeat oncoprotein that interacts with CDK4 kinase and the S6 ATPase of the 26 S proteasome. J. Biol. Chem. *277*, 10893–10902.

Dowen, S.E., Neutze, D.M., Pett, M.R., Cottage, A., Stern, P., Coleman, N., and Stanley, M.A. (2003). Amplification of chromosome 5p correlates with increased expression of Skp2 in HPV-immortalized keratinocytes. Oncogene *22*, 2531–2540.

Ellis, M., Chew, Y.P., Fallis, L., Freddersdorf, S., Boshoff, C., Weiss, R.A., Lu, X., and Mittnacht, S. (1999). Degradation of p27(Kip) cdk inhibitor triggered by Kaposi's sarcoma virus cyclin-cdk6 complex. EMBO J. *18*, 644–653.

Grasser, F.A., Graf, T., and Lipsick, J.S. (1991). Protein truncation is required for the activation of the c-myb proto-oncogene. Mol. Cell. Biol. *11*, 3987–3996.

Hainaut, P., and Hollstein, M. (2000). p53 and human cancer: the first ten thousand mutations. Adv. Cancer Res. *77*, 81–137.

Hershko, A., and Ciechanover, A. (1998). The ubiquitin system. Annu. Rev. Biochem. *67*, 425–479.

Hideshima, T., and Anderson, K.C. (2002). Molecular mechanisms of novel therapeutic approaches for multiple myeloma. Nat. Rev. Cancer *2*, 927–937.

Higashitsuji, H., Itoh, K., Nagao, T., Dawson, S., Nonoguchi, K., Kido, T., Mayer, R.J., Arii, S., and Fujita, J. (2000). Reduced stability of retinoblastoma protein by gankyrin, an oncogenic ankyrin-repeat protein overexpressed in hepatomas. Nat. Med. *6*, 96–99.

Jin, J., and Harper, J.W. (2003). A license to kill: Transcriptional activation and enhanced turnover of Myc by the SCFSkp2 ubiquitin ligase. Cancer Cell *3*, 517–518.

Kaelin, W.G., Jr. (2002). Molecular basis of the VHL hereditary cancer syndrome. Nat. Rev. Cancer *2*, 673–682.

Lai, E.C. (2002). Protein degradation: four E3s for the notch pathway. Curr. Biol. *12*, R74–R78.

LeBlanc, R., Catley, L.P., Hideshima, T., Lentzsch, S., Mitsiades, C.S., Mitsiades, N., Neuberg, D., Goloubeva, O., Pien, C.S., Adams, J., et al. (2002). Proteasome inhibitor PS-341 inhibits human myeloma cell growth in vivo and prolongs survival in a murine model. Cancer Res. *62*, 4996–5000.

Luker, G.D., Pica, C.M., Song, J., Luker, K.E., and Piwnica-Worms, D. (2003). Imaging 26S proteasome activity and inhibition in living mice. Nat. Med. *9*, 969–973.

Lynch, H.T., and de la Chapelle, A. (2003). Hereditary colorectal cancer. N. Engl. J. Med. *348*, 919–932.

Mann, D.J., Child, E.S., Swanton, C., Laman, H., and Jones, N. (1999). Modulation of p27(Kip1) levels by the cyclin encoded by Kaposi's sarcoma-associated herpesvirus. EMBO J. *18*, 654–663.

Michael, D., and Oren, M. (2002). The p53 and Mdm2 families in cancer. Curr. Opin. Genet. Dev. *12*, 53–59.

Munger, K., Basile, J.R., Duensing, S., Eichten, A., Gonzalez, S.L., Grace, M., and Zacny, V.L. (2001). Biological activities and molecular targets of the human papillomavirus E7 oncoprotein. Oncogene *20*, 7888–7898.

Peschard, P., and Park, M. (2003). Escape from Cbl-mediated downregulation. A recurrent theme for oncogenic deregulation of receptor tyrosine kinases. Cancer Cell *3*, 519–523.

Peters, J.M. (2003). Emi1 proteolysis: how SCF(beta-Trcp1) helps to activate the anaphase-promoting complex. Mol. Cell *11*, 1420–1421.

Philipp-Staheli, J., Payne, S.R., and Kemp, C.J. (2001). p27(Kip1): regulation and function of a haploinsufficient tumor suppressor and its misregulation in cancer. Exp. Cell Res. *264*, 148–168.

Pickart, C.M. (2001). Mechanisms underlying ubiquitination. Annu. Rev. Biochem. *70*, 503–533.

Polakis, P. (1999). The oncogenic activation of beta-catenin. Curr. Opin. Genet. Dev. *9*, 15–21.

Polakis, P. (2002). Casein kinase 1: a Wnt'er of disconnect. Curr. Biol. *12*, R499–R501.

Querido, E., Blanchette, P., Yan, Q., Kamura, T., Morrison, M., Boivin, D., Kaelin, W.G., Conaway, R.C., Conaway, J.W., and Branton, P.E. (2001). Degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. Genes Dev. *15*, 3104–3117.

Richardson, P. (2003). Clinical update: proteasome inhibitors in hematologic malignancies. Cancer Treat. Rev. *29* Suppl 1, 33–39.

Roberts, A., and Wakefield, L. (2003). The two faces of transforming growth factor β in carcinogenesis. Proc. Natl. Acad. Sci. USA *100*, 8621–8623.

Seagroves, T., and Johnson, R.S. (2002). Two HIFs may be better than one. Cancer Cell *1*, 211–213.

Slingerland, J., and Pagano, M. (2000). Regulation of the cdk inhibitor p27 and its deregulation in cancer. J. Cell. Physiol. *183*, 10–17.

Soldatenkov, V.A., Dritschilo, A., Ronai, Z., and Fuchs, S.Y. (1999). Inhibition of homologue of Slimb (HOS) function sensitizes human melanoma cells for apoptosis. Cancer Res. *59*, 5085–5088.

Spruck, C.H., Strohmaier, H., Sangfelt, O., Muller, H.M., Hubalek, M., Muller-Holzner, E., Marth, C., Widschwendter, M., and Reed, S.I. (2002). hCDC4 gene mutations in endometrial cancer. Cancer Res. *62*, 4535–4539.

Treier, M., Staszewski, L., and Bohmann, D. (1994). Ubiquitin-dependent c-Jun degradation in vivo is mediated by the ∂ domain. Cell *78*, 787–798.

Vandenberg, C., Gergely, F., Yi Ong, C., Pace, P., Mallery, D., Hiom, K., and Patel, K. (2003). BRCA1-independent ubiquitination of FANCD2. Mol. Cell *12*, 247–254.

Wang, J., Chenivesse, X., Henglein, B., and Brechot, C. (1990). Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. Nature *343*, 555–557.

Wang, J., Zindy, F., Chenivesse, X., Lamas, E., Henglein, B., and Brechot, C. (1992). Modification of cyclin A expression by hepatitis B virus DNA integration in a hepatocellular carcinoma. Oncogene *7*, 1653–1656.

Wang, W., Takimoto, R., Rastinejad, F., and El-Deiry, W.S. (2003). Stabilization of p53 by CP-31398 inhibits ubiquitination without altering phosphorylation at serine 15 or 20 or MDM2 binding. Mol. Cell. Biol. *23*, 2171–2181.

Wilkinson, K.D. (2000). Ubiquitination and deubiquitination: targeting of proteins for degradation by the proteasome. Semin. Cell Dev. Biol. *11*, 141–148.

Yokoi, S., Yasui, K., Saito-Ohara, F., Koshikawa, K., Iizasa, T., Fujisawa, T., Terasaki, T., Horii, A., Takahashi, T., Hirohashi, S., and Inazawa, J. (2002). A novel target gene, SKP2, within the 5p13 amplicon that is frequently detected in small cell lung cancers. Am. J. Pathol. *161*, 207–216.