The ubiquitin–proteasome pathway in cancer

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Summary Degradation by the 26S proteasome of specific proteins that have been targeted by the ubiquitin pathway is the major intracellular non-lysosomal proteolytic mechanism and is involved in a broad range of processes, such as cell cycle progression, antigen presentation and control of gene expression. Recent work, reviewed here, has shown that this pathway is often the target of cancer-related deregulation and can underlie processes, such as oncogenic transformation, tumour progression, escape from immune surveillance and drug resistance.

Keywords: ubiquitin; proteasome; oncogenesis; drug resistance; immune escape

Eukaryotic cells contain two major proteolytic pathways, namely the lysosomal pathway, which mainly degrades extracellular proteins that have entered the cell via endocytosis or pinocytosis, and the non-lysosomal pathway, which degrades in a cellular particle called the proteasome intracellular proteins, which have been targeted for destruction by a protein called ubiquitin. The ubiquitin–proteasome pathway was initially regarded as a simple mechanism of destruction for old or damaged proteins, but it is now emerging as a crucial mechanism in cellular regulation. Indeed, in recent years it has been found that protein degradation accounts for the regulation of proteins, such as cyclins, cyclin-dependent kinase inhibitors, p53, c-JUN and c-FOS, and it has become increasingly clear that proteolysis is a mechanism of regulation of many cellular processes, including cell cycle progression, transcriptional regulation and antigen presentation (Hochstrasser, 1995; King, 1996; Pahl and Baueuerle, 1996). The importance of proteolysis probably stems from the advantages that it offers over other regulation mechanisms, such as the rapidity of the reduction of the cellular level of a specific protein and the irreversibility of the loss of function after degradation. In addition, it has to be stressed that, by degrading inhibitors or activators of the various pathways, protein degradation can act both as an up-regulation or a down-regulation mechanism.

The ubiquitin–proteasome pathway degrades cytosolic and nuclear proteins via an ATP- and ubiquitin-dependent mechanism, which is centred on a multicatalytic proteasome complex called the 26S proteasome. Substrate proteins are targeted for degradation by the addition of multiple monomers of ubiquitin, a 76 amino acid polypeptide, to specific residues in a multi-step reaction requiring three classes of enzymes called E1, E2 and E3. Initially, a ubiquitin-activating enzyme (E1) activates a ubiquitin monomer at its C-terminal glycine residue to a high-energy thiol ester intermediate. Then, E2 enzymes, also known as ubiquitin-conjugating enzymes (UBC), transfer ubiquitin from E1 to the substrate that is bound to a ubiquitin-protein ligase (E3). The first ubiquitin molecule is usually bound to the substrate by an isopeptide bond between the C-terminal glycine of ubiquitin and an ε-NH₂ group of a lysine residue of the substrate. The polyubiquitin chain is formed in multiple cycles of this reaction by addition of another ubiquitin molecule to the lysine at position 48 of the previously already conjugated ubiquitin. Release of ubiquitin from the isopeptide linkage with the lysine residue is performed by isopeptidases called ubiquitin C-terminal hydrolases (UCH). Their function is probably important not only in recycling ubiquitin monomers after substrate degradation but also in the recovery of poorly or incorrectly ubiquitinated proteins (Shafer and Cohen, 1996).

Polyubiquitinated proteins are substrates for the 26S proteasome. This consists of three large multi-subunit complexes, namely a 700-kDa 20S proteasome core particle and two 19S cap structures, also called PA 700 (for proteasome activator of 700 kDa) (reviewed in Peters, 1994). The 20S particle has the structure of a hollow cylinder composed of four rings of seven related subunits and containing a central channel with three cavities (Löwe et al, 1995; Groll et al 1997). The inner rings are formed of β-subunits, which carry the proteolytically active sites on the inner surface. The outer rings contain subunits that lack proteolytic activity and are thought to control the access to the central cavity. The isolated 20S particle has very limited activity in vitro compared with the 26S proteasome, which is formed by the 20S proteasome with the addition of two 19S/PA700 substructures in opposite orientations, one at each end (Peters et al, 1993), as revealed by electron microscopy (Figure 1). The 19S regulatory complex consists of at least 15 subunits, which can be classified into ATPases and non-ATPases (Dubiel et al, 1995a), and is thought to act in recognition, unfolding and translocation of the substrates into the 20S proteasome for proteolysis (Rubin and Finley, 1995). The composition and the function of the regulatory complex is not yet fully characterized and recent data have shown, for example, that the regulatory complex also contains an isopeptidase capable of deubiquitinating substrates (Lam et al, 1997).

Because of the broad involvement of ubiquitin–proteasome proteolysis in fundamental biochemical processes, this pathway is a potential target for cancer-related deregulation, and alterations of proteasome function have indeed been described in events, such as cellular transformation by oncogenic viruses (Scheffner et al, 1997).
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The ubiquitin–proteasome pathway is a prototypical example of a multienzymatic cellular regulatory cascade. It is involved in controlling the turnover of proteins by catalyzing their degradation by the 26S proteasome. The pathway has a central role in a variety of cellular functions, including cell cycle control, DNA damage response, and apoptosis. This pathway is particularly important in cancer biology, where it plays a critical role in the regulation of cell cycle progression, apoptosis, and response to DNA damage. The pathway is often disrupted in cancer cells, leading to alterations in protein homeostasis and contributing to the malignant phenotype.

**Figure 1** (Adapted with permission from Rubin and Finley, 1995). The 26S proteasome is a multiprotein complex that acts as a multicatalytic protease degrading proteins that have been targeted by the ubiquitin pathway. Proteins are ubiquitinated in a cascade reaction involving three classes of ubiquitinating enzymes called E1, E2 and E3 and can be deubiquitinated by isopeptidases. The 20S proteasome consists of a stack of four rings of seven subunits. The inner rings made of β-subunits display the catalytic sites on the inner surface. At each end, the 20S proteasome can be capped by a regulatory complex called 19S or PA700, which contains ATPases and is probably involved in recognition, unfolding and translocation of the substrate into the 20S proteasome (Rubin and Finley, 1995).

1990, 1993; Ciechanover et al, 1994) and immune escape (Restifo et al, 1993; Sibille et al, 1995; Rotem Yehudar et al, 1996; Seliger et al, 1996). Furthermore, alterations of proteasome activity in tumour samples have been reported recently to confer in colon and possibly breast cancer a phenotype of clinical aggressiveness associated with poor prognosis (Catzavelos et al, 1997; Loda et al, 1997; Porter et al, 1997). Finally, mutations of proteasome subunits have been found to result in a multidrug resistance phenotype in fission yeast (Gordon et al, 1993, 1996), and we have recently shown that this pathway of multidrug resistance is conserved in mammalian cells (Spataro et al, 1997). Here, we therefore review the rapidly increasing body of information on the role of proteolysis by the ubiquitin/proteasome pathway in various fields of cancer biology.

**p27 AS A PROGNOSTIC FACTOR**

Progression through the cell cycle is promoted by oscillation in the activity of cyclin-dependent kinases (CDK), and proteolysis by the ubiquitin–proteasome pathway regulates CDK activity by degrading CDK activators and inhibitors. Furthermore, proteolysis by the proteasome is crucial during mitosis in triggering the transition from metaphase to anaphase (reviewed in King, 1996). Among the substrates for proteolysis in the cell cycle machinery, clinically important data are emerging with regard to the CDK inhibitor p27. p27 inhibits a wide variety of cyclin–CDK complexes in vitro and its activity is up-regulated by cytokines, such as TGF-β and by cell–cell contact, linking extracellular signals to the cell cycle (Polyak, 1994; Slingerland, 1994). Loss of contact inhibition and of response to TGF-β in transformed cells may imply an alteration of function of p27 during oncogenesis, even though p27 mutations in human tumours are extremely rare (Hunter and Pines, 1994; Morosetti et al, 1995; Ferrando, 1996). Unlike p21, which is also a member of the family of cip/kip CDK inhibitors acting in G1, and appears to be regulated principally at the transcriptional level, p27 is critically regulated post-translationally by proteolysis by the ubiquitin–proteasome pathway (Pagano et al, 1995; Hengst and Reed, 1996). Recently, it has been found that low p27 protein levels in common tumours, such as colorectal carcinomas and breast cancer, are associated with a poor prognosis (Catzavelos et al, 1997; Loda et al, 1997; Porter et al, 1997). In both tumour types (Catzavelos et al, 1997; Loda et al, 1997), comparison of immunohistochemical analysis and in situ hybridization showed a discordance between p27 mRNA and protein levels, suggesting that, also in tumours, p27 levels could be regulated post-translationally. Moreover, in one of the studies, it was clearly shown that increased proteasome-dependent degradation was responsible for low p27.

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Table 1 Summary of settings in which the ubiquitin–proteasome pathway plays a role in cancer (see text for references)

| Substrate                              | Proteolysis dysregulation | Functional effect                      | Biological effect                              | Comment                                                                 |
|----------------------------------------|---------------------------|---------------------------------------|------------------------------------------------|------------------------------------------------------------------------|
| p53                                    | Increased                 | p53 inactivation                      | Transformation in HPV-related malignancies    | Mediated by E6 oncoprotein of 'high-risk' HPV                           |
| p27                                    | Increased                 | p27 inactivation                      | Tumour progression                            | Unfavourable prognosis in retrospective studies in colon and breast cancer |
| Cyclin D1, E and B                     | Decreased?                | Cyclin D1, E or B overexpression      | Tumour progression                            | Overexpression in tumour cell lines and surgical specimens, contribution of decreased degradation? |
| MHC-I-restricted                       | Decreased                 | Defective antigen presentation        | Escape from immune surveillance              | Suggested by in vitro studies, preliminary in vivo evidence             |
| NF-κB inhibitor, κB                    | Increased                 | Increased NF-κB activation            | Resistance to TNF-α killing                  | Overcome by proteasome inhibitors                                      |
| Transcription factors of the AP-1 family | Decreased                 | Increased AP-1 activity               | Multidrug resistance                          | Evidence in yeast, highly conserved pathway in human, role in tumour drug resistance? |

levels in tumour samples of colorectal carcinomas. Total cellular extracts from frozen tumour samples were tested for p27 proteasome-mediated degradation using recombinant p27 as a substrate, and a very good correlation was found between low levels of p27 and increased proteasome activity. Degradation was abolished by proteasome depletion and resumed after proteasome readdition (Loda et al, 1997). Down-regulation of p27 by the proteasome was found in tumours regardless of clinical stage. In breast cancer, Catzavelos et al (1997) showed that increased p27 proteolysis can be an early event in tumorigenesis, as suggested by analysis of high-grade ductal carcinoma in situ (DCIS) or, alternatively, can occur upon progression, as shown by reduced p27 levels in axillary lymph node metastasis compared with primary tumours assessed simultaneously. Even taking into account the caveats associated with retrospective studies on prognostic factors, these three recent studies (Catzavelos et al, 1997; Loda et al, 1997; Porter et al, 1997) conclude that p27 protein level (and its proteasome-dependent degradation, which was shown to be inversely related) are powerful independent prognostic factors of survival in both tumour types and show clearly that deregulation of gene products involved in clinical tumour progression can occur via alterations of ubiquitin–proteasome proteolysis. Furthermore, they show that this is not a rare event, given that the unfavourable phenotype of decreased p27 levels (defined as immunostaining in < 50% of the cells or as a score of staining of 0–1 on a 0 to 6 scale) involves the majority of the studied population for both colorectal cancer and breast cancer (Catzavelos et al, 1997; Loda et al, 1997; Porter et al, 1997).

Thus the frequency of the phenotype of decreased p27 and its distribution, which is independent of most other prognostic factors, make p27 levels a very promising new prognostic factor to be evaluated further. Loda et al (1997) have shown that, perhaps unexpectedly, p27 degradation activity is not correlated with degradation by the proteasome of other substrates, such as p21 and cyclin A, which underscores that the substrate specificity of the ubiquitin–proteasome pathway is highly regulated (Hochstrasser, 1995). Identification of the element(s) responsible for targeting p27 to the ubiquitin–proteasome pathway would, of course, be extremely important for unravelling this novel pathway associated with tumour progression.

Like p27, other elements of the cell cycle machinery that are substrates of ubiquitin–proteasome degradation are potential targets for deregulation in tumours. One of the best characterized transitions in the normal cell cycle is the rapid proteasome-mediated degradation of cyclin B at the exit from mitosis (Glotzer et al, 1991), and recent evidence shows that continuing rapid proteolysis accounts for the low levels of cyclin B until the onset of S phase (Amon et al, 1994; Brandeis, 1996). Cyclin B has been found to be overexpressed in a set of breast cancer cell lines (Keyomarsi and Pardee, 1993), and it would be interesting to assess whether or not decreased proteolysis by the proteasome is involved in its overexpression. Similarly, cyclin E has been found to be overexpressed in breast cancer cell lines and in surgical specimens of breast tumours (Keyomarsi et al, 1994, 1995), and cyclin D1 is frequently overexpressed in many common tumour types (Betticher, 1996). Recent evidence suggests that cyclin D1 and E are substrates of the ubiquitin–proteasome pathway (Clurman et al, 1996; Diehl et al, 1997), and decreases in their degradation could contribute to the overexpression of these cyclins in tumours.

**ANTIGEN PRESENTATION**

The 26S proteasome is responsible for the processing of MHC-restricted class I antigens. Peptides derived from endogenously expressed cytoplasmic proteins are carried by MHC class I molecules from the endoplasmic reticulum to the surface for recognition by cytotoxic T lymphocytes. The proteasome was postulated to be the proteolytic system that degrades cytosolic proteins, when it was found that the genes encoding subunits LMP-2 and LMP-7 of the
proteasome complex were included in the MHC gene cluster (see for example Beck et al, 1992). Experiments performed in a mutant cell with a thermolabile El-ubiquitinating enzyme (Michalek et al, 1993) and with proteasome inhibitors (Rock et al, 1994; Cerundolo et al, 1997) have subsequently demonstrated that the proteasome is necessary for class I-restricted antigen presentation. This is confirmed by the analysis of mice lacking LMP-7, which have decreased surface expression of MHC class I molecules and present antigens inefficiently (Fehling et al, 1994). It has also been shown that 3 of the 28 subunits composing the 20S catalytic core, namely subunits X, Y and Z, are interchangeable with the alternative subunits LMP2, LMP7 and LMP10 respectively (Belich et al, 1994; Fruh et al, 1994; Groettrup et al, 1996; Hisamatsu et al, 1996; Nandi et al, 1996) upon induction by interferon-γ. These substitutions result in an enhancement of peptide activity, a change in the quality of generated peptides (Gaczyńska et al, 1996; Kuckelkorn et al, 1995) and eventually in a more efficient antigen presentation. Interferon-γ also induces the binding to the 20S catalytic core of the proteasome of a complex called 11S regulator or PA28, which may further increase the spectrum of peptides generated (Groettrup et al, 1995). There is strong evidence that MHC class I-restricted peptide presentation is modified in tumours and may contribute to escape from immune surveillance. Alterations of ubiquitin–proteasome degradation have been reported among other alterations in this pathway. Three different small-cell lung carcinoma lines with low to undetectable levels of mRNA for LMP2 and LMP7 and functional deficiencies in antigen presentation have been described (Restifo et al, 1993). The mouse T-cell lymphoma line SP-3 displays underexpression of LMP-2 and is defective for antigen presentation, whereas LMP-2 expression and antigen presentation to cytotoxic T lymphocytes are restored upon expression of interferon-γ by transfection (Sibille et al, 1995). Similar studies on tumour samples are rare. An analysis of expression of both LMP-2 and LMP-7 proteasome subunits together with the antigen presentation machinery has been carried out on a primary renal cancer and a lymph node metastasis of the same patient and compared with normal kidney. Deficiencies at all levels, including the expression of LMP-2 and LMP-7 proteasome subunits, were associated with transformation and progression. Interferon-α and, in particular, interferon-γ could partly suppress these defects (Seliger et al, 1996). The potential importance of subunits LMP-2 and LMP-7 for MHC class I-restricted antigen presentation is also underscored by the fact that they are specifically down-regulated after viral transformation in vitro by oncogenic viruses (Rotem Yehudar et al, 1996).

REGULATION OF TRANSCRIPTION FACTORS BY PROTEOLYSIS

Increasing evidence shows that the proteasome also participates in events that control gene transcription. Several transcriptional regulators, including nuclear factor-kappa B (NF-κB), p53 (see above), c-JUN, sterol-regulated element-binding proteins and MATα2 have been recently shown to be regulated by proteolysis, either for the activation or the inactivation of gene expression (for a review see Pahl and Baeuerle, 1996).

NF-κB is involved in the activation of genes encoding products such as cytokines, chemokines, growth factors, cell-adhesion molecules and surface receptors in response to a great variety of pathogenic signals and therefore has a central role in mediating the immune/inflammatory responses. NF-κB has been reported to be

activated by the cytotoxic agents TNF-α, daunorubicin, etoposide, ionizing radiation or oxidative stress but not by the protein kinase C inhibitor staurosporine (Wang et al, 1996). The activation of NF-κB requires two steps of proteasome-dependent proteolysis. Active NF-κB is a nuclear heterodimer consisting of two subunits called p50 and p65. Ubiquitin–proteasome proteolysis is involved first in the biogenesis of the subunit p50 from the precursor p105 and then in the cytoplasmic degradation of the inhibitory factor IκB, which allows the translocation of the active dimer into the nucleus (Palombella et al, 1994). Recently published data attribute an anti-apoptotic role to NF-κB in response to some cytotoxic agents (Beg and Baltimore, 1996; Van Antwerp et al, 1996; Wang et al, 1996). In one case, TNF-α was more toxic for immortalized embryonic cells of NF-κB knock-out mice than for controls (Beg and Baltimore, 1996) and in other experiments expression of the super-repressor IκB-a (inhibiting NF-κB activation) moderately increased the sensitivity to TNF-α, daunorubicin and ionizing radiation (Wang et al, 1996). Consistent with this, the proteasome inhibitor MG132 (preventing NF-κB activation) strongly enhanced, in a dose-dependent fashion, the killing of HT1080V cells by TNF-α. The prote-oxonogenic products c-JUN and c-FOS constitute the transcription factor AP-1 (for activator protein 1) either as heterodimers or as c-JUN homodimers and are well-known substrates for ubiquitin–proteasome degradation (Treier et al, 1994; Jariel Encontre et al, 1995; Tsurumi et al, 1995a; Hermida Matsumoto et al, 1996; Musti et al, 1997). The degradation of c-JUN is dependent on a segment of 27 amino acids called the delta domain, which is necessary for both ubiquitination and degradation. The delta region, and hence this mechanism of down-regulation, is lost in v-JUN, the transforming retroviral counterpart of c-JUN, and this increased stability very likely contributes to its oncogenicity (Treier et al, 1994). Moreover, it has been convincingly shown that c-JUN is degraded by this pathway, but recent data suggest that ubiquitin–proteasome-mediated proteolysis of c-JUN could play an essential role in regulation of activity of AP-1 factors (Musti et al, 1997). There is a high degree of regulation of c-JUN proteolysis, with the presence of c-FOS and dimerization itself influencing the ubiquitination and the degradation activity (Tsurumi et al, 1995a; Hermida Matsumoto et al, 1996). Like NF-κB, AP-1 factors are important in the cellular response to oxidative stress (Schreiber et al, 1995; Pinkus et al, 1996) and are involved in the induction of a variety of genes encoding important enzymes in glutathione-related detoxification pathways, such as the isozymes α, π and γ of glutathione-S-transferase and γ-glutamyl-cysteine synthetase. Up-regulation of AP-1 activity has been associated with drug resistance in several instances, such as in a multidrug-resistant derivative of MCF7 cells obtained after vincristine selection (Moffat et al, 1994) in etoposide-resistant human leukaemia cell lines (Ritke et al, 1994) and in cisplatin-resistant ovarian cancer lines (Yao et al, 1995). Given the relevance of proteolysis for c-JUN regulation, this acquires particular importance in the light of recent data discussed in the following section that link the proteasome, AP-1 factors and multidrug resistance (Spataro et al, 1997).

DRUG RESISTANCE

We recently identified a novel component of the 26S proteasome that indicates a link between ubiquitin-dependent proteolysis and drug resistance. Overexpression of the fission yeast Pad1 protein confers multidrug resistance to unrelated compounds, such as
staurosporine, caffeine and leptomycin B, through the activation of the yeast transcription factor Pap1, a homologue of human AP-1 (Shimanuki et al, 1995). Because studies in yeast may help to identify important novel mechanisms in mammalian cells, we set out to examine the role of a Pad1 human homologue. We have cloned the human homologue of Pad1 (named POH1 for Pad One Homologue) and have shown by transfection experiments that its overexpression in mammalian cells can confer multidrug resistance to 7-hydroxystaurosporine, paclitaxel, doxorubicin and to ultraviolet radiation. Interestingly, the amino acid sequence of POH1 displayed a significant similarity to the subunit S12/p40 of the 26S proteasome (Dubiel et al, 1995b; Tsurumi et al, 1995b), and the pattern of mRNA tissue expression was very similar to that previously described for other subunits of the 26S proteasome (Tsurumi et al, 1995b). We demonstrated that POH1 is in fact a novel subunit of the 26S proteasome, as it co-purifies with proteasome immunoprecipitates and with full 26S proteasomes obtained by biochemical fractionation (Spataro et al, 1997). POH1 also has a significant sequence similarity with JAB1, which has been shown to interact with c-JUN and to activate AP-1 transcription factors (Claret et al, 1996). Various independent data, namely the dependence of the pad1 multidrug resistance phenotype in fission yeast on the activation of an AP-1 like factor, the sequence similarity between POH1 and JAB1 and the importance of proteasome degradation for c-JUN regulation support a model whereby over-expression of the novel proteasome subunit POH1 could up-regulate AP-1 factors, resulting ultimately in drug resistance. Our data show that POH1 overexpression does not activate P-glycoprotein expression and does not alter intracellular accumulation of doxorubicin. Nevertheless, it is not clear at this stage if the survival advantage conferred by POH1 overexpression reflects a decreased propensity for cell death or an alteration in the processing of potentially lethal damage. POH1 is widely expressed in human tumour cell lines and work in progress is assessing its contribution to tumour drug resistance. Interestingly, in recent years, two other subunits of the 19S regulatory complex of the proteasome called Mts2 and Mts3 have been identified in fission yeast through a screen for mutants resistant to the mitotic spindle poison carbendazim (MBC) (Gordon et al, 1993, 1996). Thus, the 26S proteasome plays an important role in determining multidrug resistance in fission yeast. This pathway is highly conserved in mammals, can confer drug resistance to anti-cancer agents in vitro and could potentially be involved in drug resistance in human tumours. A human homologue of another fission yeast gene called Cmn1, which is involved like Pad1/POH1 in Pap1/AP-1-dependent multidrug resistance (Toda et al, 1992; Kumada et al, 1996) has been recently cloned (Fornerod et al, 1997). Interestingly, its protein product interacts with the DEK-CAN fusion protein of AML with the chromosomal translocation t(6;9), which is associated with poor prognosis (Lillington et al, 1993). It is possible that proteasome/AP-1-mediated drug resistance contributes to the dismal prognosis of this uncommon subset of acute myeloid leukaemia (AML).

OTHER AREAS OF CANCER BIOLOGY

Among other areas of cancer biology in which involvement of the ubiquitin–26S proteasome pathway may be relevant, growth factor receptors and their signalling pathways should not be overlooked. Several cell-surface receptors have been shown to be ubiquitinated, suggesting that proteasome-mediated proteolysis could be involved in their turnover (for a list see Ciechanover, 1994). Involvement of proteasomes in the degradation of cell surface receptors might have an increasing relevance in cancer chemotherapy, as new agents that modulate growth factors and their signalling pathways are developed. For example, there is strong evidence for an involvement of the ubiquitin–proteasome pathway in the degradation of tyrosine kinase receptors, such as insulin-like growth factor receptors and epidermal growth factor receptors. Of interest, it has been shown recently that herbimycin A, which targets tyrosine–kinase-activated signal transduction by inhibiting multiple tyrosine protein kinases and has in vitro and in vivo anti-tumour activity, acts through an enhancement of receptor degradation by the proteasome (Sepp Lorenzino et al, 1995). Similar data have also been found with regard to the partly agonist protein kinase C (PKC) inhibitor bryostatin 1 (Philip and Harris, 1995), which after transient activation down-regulates PKC through the promotion of its degradation by the proteasome (Lee et al, 1996). Proteasome inhibitors have also been shown to counteract the effects of herbimycin A in vitro (Sepp Lorenzino et al, 1995), and it is conceivable that modulation of proteasome function might influence the anti-tumour activity of these new classes of drugs.

Other cell surface receptors that are potential targets for proteasome degradation are the T-cell antigen receptor (TCR) and the platelet-derived growth factor (PDGF) receptor. One T-cell receptor subunit is ubiquitinated on its cytoplasmic domain when the receptor is occupied (Hou et al, 1994), but data are lacking on possible effects on its function. The PDGF receptor-β also undergoes polyubiquitination as a consequence of ligand binding and, recent data suggest that the proteasome is responsible for the degradation of the ligand-activated receptor (Mori et al, 1995).

DNA repair is another important area in which the ubiquitin–proteasome pathway is potentially involved. The first data supporting this notion came from budding yeast S. cerevisiae, in which the rad6 DNA repair mutant is defective in the ubiquitin-conjugating enzyme (E2) UBC2 and, intriguingly, the DNA repair gene RAD23 encodes a protein containing a ubiquitin-like domain, which is essential to its function (Watkins et al, 1993) and is conserved in the human homologue HHR23B (Masutani et al, 1994). More recently, experiments performed on a ts mutant from the mouse mammary carcinoma line FM3A, which contains a thermosensitive ubiquitin-activating enzyme (E1) have shown that E1 mutants incubated at the restrictive temperature after UV exposure display a decrease in clonogenic survival and defects in an assay measuring DNA repair by the appearance of UV-induced mutations (Ikehata et al, 1997). These data support a contribution of ubiquitin conjugation to DNA repair in mammalian cells. However, it remains to be seen if there is a true contribution to DNA repair of the entire pathway of ubiquitin–proteasome-mediated proteolysis or if, alternatively, ubiquitin-binding proteins, such as E1 or E2 enzymes, may have a direct influence on DNA repair by physically interacting with DNA repair proteins carrying ubiquitin-like domains, such as RAD23/HHR23B. Another area where intriguing data awaits further elucidation is the potential role of deubiquitinating enzymes in oncogenic transformation, as the yeast DOA4 isopeptidase is related to the product of the human Tre-2, which has been found to be tumorigenic when expressed at high levels (Papa and Hochstrasser, 1993); in addition, the human homologue of the murine ubiquitin-releasing enzyme ulp has been found to be over-expressed in lung cancer cell lines (Gray et al, 1995).

Recently, ubiquitin–proteasome-mediated proteolysis has also been found to have an important role in apoptosis of nerve growth
factor-deprived neurons (Sadoul et al, 1996), and it will be important to investigate proteasome involvement in apoptosis induced by anti-cancer drugs. Finally, it has recently been shown that expression of heat shock protein 70 (hsp70), which is involved in stress response and might have a role in drug resistance (Ciocca et al, 1992), is induced up to 30-fold by a proteasome inhibitor, unlike other members of the hsp family (Zhou et al, 1996).

DRUGS ACTING ON THE PROTEASOME

Pharmacological intervention to modulate one or several proteasome functions could be therapeutically advantageous. There is considerable interest in this possibility in the field of immunology, in which the intent is to target activation by the proteasome of NF-κB, which has a key role in mediating the inflammatory and immune response. The best known proteasome inhibitor is lactacystin, a Streptomyces metabolite discovered on the basis of its ability to induce neurite outgrowth in the Neuro 2A mouse neuroblastoma cell line (Fenteany et al, 1994). This inhibitor was subsequently shown to covalently modify a critical threonine residue of the subunit X/MB1 of the proteasome core (Fenteaney, 1995). Lactacystin was found to inhibit cell cycle progression in human osteosarcoma cells (Fenteany et al, 1994) and to induce apoptosis in human monoblast cells (Imajoh Ohmi et al, 1995). However, we are not aware of any data on the anti-tumour activity of lactacystin. Interestingly, the clinically used anti-tumour drug aclacinomycin A or aclacinubicin, known as a DNA-intercalating agent, has been shown to inhibit the degradation of ubiquitinated protein by selectively inhibiting the chymotrypsin-like activity of the proteasome (Figueiredo Pereira et al, 1996). It is not clear whether this could contribute to the anti-tumour activity of this drug. Apart from lactacystin, most of the proteasome inhibitors developed so far are synthetic protease inhibitors of the family of peptidyl aldehydes (Rock et al, 1994). Some of them, such as N-acetyl-leucinyl-leucinyl-norleucinal (ALLN) and benzoyloxycarbonyl (Z)-leucinyl-leucinyl-leucinal (ZLLL) are cell penetrating, display proteasome specificity and have been reported to induce apoptosis in human tumour cell lines (Fujita et al, 1996; Shinohara et al, 1996). Because of the broad involvement of proteasomes in normal cellular physiology, any attempt to target the proteasome non-specifically might be associated with prohibitive in vivo toxicity. However, the complexity and specificity of proteasome regulation indicate that specific inhibitors of individual proteasome-mediated processes might ultimately become available. Moreover, the rapidly expanding knowledge about the role of proteasomes in normal and tumour cells could provide in the future a rational basis for the use of proteasome-targeting drugs.

CONCLUSIONS

The ubiquitin–proteasome pathway clearly represents an important area of research in cancer biology, although it has previously been relatively neglected. Basic research has provided in recent years an increasing body of information on the extent of the involvement of this pathway in critical cellular processes, such as cell cycle progression and regulation of gene expression. To date, research has found that deregulation of this pathway in cancer can be responsible for crucial phenomena, such as oncogenic transformation in HPV-related malignancies, poor prognosis in colorectal and breast carcinoma, and that it is clearly involved in modulating response to anti-cancer drugs. Understanding the complexity of the ubiquitin–proteasome pathway, and in particular how the specificity for a given substrate is regulated, should allow us in the future to translate this knowledge into new therapeutic strategies.

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