EUROPEAN JOURNAL OF CANCER 43 (2007) 2330–2338

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Review

The putative roles of the ubiquitin/proteasome pathway in resistance to anticancer therapy

*Laura Smith, Michael J. Lind, Philip J. Drew, Lynn Cawkwell**

Cancer Biology Proteomics Group, Postgraduate Medical Institute of the University of Hull in association with the Hull-York Medical School, University of Hull, UK

ARTICLE INFO

Article history:
Received 16 May 2007
Received in revised form 9 July 2007
Accepted 18 July 2007
Available online 20 September 2007

Keywords:
Proteasome
Ubiquitin
Chemotherapy
Radiotherapy

ABSTRACT

The ubiquitin/proteasome (UP) pathway plays a significant role in many important biological functions and alterations in this pathway have been shown to contribute to the pathology of many human diseases, including cancer. Proteasome inhibition has been well established as a rational strategy for the treatment of multiple myeloma and is currently under investigation for the treatment of other haematological malignancies and solid tumours. Recent evidence suggests that proteasome inhibition may also sensitise tumour cells to the actions of both conventional chemotherapy and radiotherapy, suggesting that this pathway may modify clinical response to anticancer therapy. However, conflicting evidence exists as to the roles of the UP pathway in resistance to treatment. This review endeavours to discuss such roles.

1. Introduction

The degradation of cellular proteins is a highly complex, temporally controlled and tightly regulated process that plays a major role in a variety of biological pathways, including cell proliferation and apoptosis. The ubiquitin/proteasome (UP) pathway is the main non-lysosomal route for degradation and is responsible for the turnover of >80% of cellular proteins.[1] This pathway involves the conjugation of multiple ($\geqslant$4) ubiquitin moieties to a substrate followed by the downstream degradation of the tagged protein by a multi-catalytic proteasome complex. Ubiquitin (Ub) is a small protein comprising 76 amino acids that becomes conjugated to target substrates via a multi-enzyme cascade consisting of Ub-activating (E1), Ub-conjugating (E2) and Ub-ligating (E3) enzymes,[2] which act in a sequential manner (Fig. 1a). Firstly, Ub becomes activated through the action of an E1 enzyme, which hydrolyses ATP and adenylates the C-terminus of Ub. A thioester bond is formed between the C-terminus of the activated Ub and a cysteine residue within the active site of the E1 enzyme. Activated Ub is then transferred to an E2 enzyme in an ATP-dependent trans-esterification reaction. This results in the formation of a further thioester bond between the activated Ub molecule and a cysteine residue within the active site of the E2 enzyme. Subsequently, an E3 enzyme mediates the transfer of the activated Ub molecule to a substrate either directly by creating an isopeptide linkage between a glycine residue located at the C-terminus of Ub and a lysine residue of the substrate or indirectly via the formation of a third thioester intermediate.[3] Several hundred mammalian E3 enzymes exist, which provide substrate specificity. De-ubiquitinating (DUB) enzymes add a further level of regulation to this system and act to cleave Ub molecules from protein substrates.[4] Additional activated Ub molecules may

* *Corresponding author:* Present address: R&D Building, Castle Hill Hospital, Hull HU16 5JQ, UK. Tel.: +44 1482 875875x3617; fax: +44 1482 622398.
E-mail address: L.Cawkwell@hull.ac.uk (L. Cawkwell).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2007.07.023

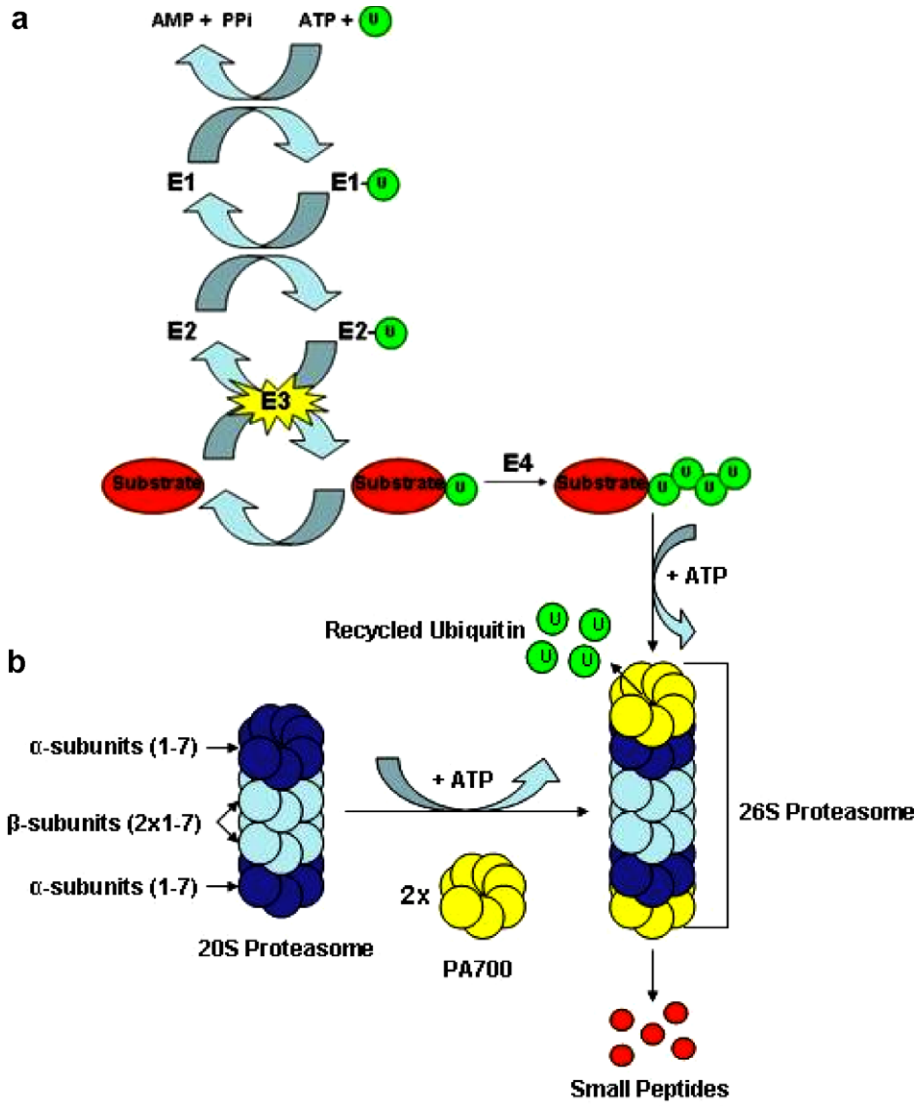

Fig. 1 – The ubiquitin/proteasome (UP) pathway. (a) The multi-enzyme cascade regulating ubiquitin conjugation to target substrates. For further information, see text. (b) The 26S proteasome. This multi-catalytic protease consists of a barrel-shaped proteolytic core called the 20S proteasome, which is composed of four stacked rings. Each outer ring contains seven α subunits (1–7) and each inner ring contains seven beta subunits (1–7). In association with PA700 (19S regulatory) complexes the 26S proteasome is formed, which is responsible for the degradation of poly-ubiquitinylated proteins (U, ubiquitin).

also be linked to the substrate via isopeptide linkages to the lysine residues of the already conjugated Ub molecules. This leads to the formation of a poly-Ub chain and is mediated by a particular type of E3 enzyme sometimes referred to as an Ub-elongation enzyme or an E4 enzyme.[2,5]

There are seven lysine residues within the Ub molecule that can be used to link them together. The destiny of the substrate protein is determined by the position at which the poly-Ub chain is linked. For example, Lys(11)-, Lys(29)- and Lys(48)-target the substrate for degradation by the 26S proteasome and Lys(6)- or Lys(63)- (as well as mono-ubiquitinylation), signal reversible modifications in protein activity, location or trafficking.[6]

Ubiquitinylated proteins are recognised and degraded by the 26S proteasome. The catalytic heart of the 26S proteasome is a barrel-shaped core called the 20S proteasome.[7,8] The 20S proteasome is composed of four stacked homomeric rings. Each outer ring contains seven distinct α subunits (1–7) encoded by the PSMA genes and each inner ring contains seven distinct beta subunits (1–7) encoded by the PSMB genes. Each ring of beta subunits contains three proteolytic sites, which differ in substrate specificity. The 'chymotrypsin-like' site (PSMB5 subunit) cleaves peptide bonds preferentially after hydrophobic residues. The 'trypsin-like' site (PSMB2 subunit) cleaves mainly after basic residues. The 'caspase-like' site (PSMB1 subunit) cleaves preferentially after acidic residues. Substrates access these active sites through the α-annulus, which is a constriction in the outer rings of the α subunits.[9] The N-terminal residues of the α subunits form a gate to the α-annulus sealing the entrance. Thus, in isolation the 20S proteasome is dormant preventing the untimely demise of important regulatory proteins and therefore requires

activation by proteasome activator complexes including PA700 (also known as the 19S regulatory complex), PA28 and PA200.[10] PA700 is the best characterised of these complexes and binds to both ends of the 20S core to form the 26S proteasome (Fig. 1b). The biochemical functions of the PA700 complex include recognising ubiquitinylated substrates, cleaving the Ub molecules from substrates for recycling and unfolding and presenting substrates to the proteolytic core of the 20S proteasome for degradation.[11]

In contrast, the PA28 and PA200 proteasome activator complexes have been shown to activate the 20S proteasome against model peptide substrates and do not recognise ubiquitinylated proteins.[10] PA28 (11S) is composed of 7 subunits of ~28-kDa each, which are encoded by the PA28α (also known as PSME1), PA28β (PSME2) and PA28γ (PSME3) genes. PA28α and PA28β subunits form a heteroheptamer, whereas PA28γ forms a homoheptamer. The PA28αβ complex binds to the ends of the 20S core activating the cleavage of peptides for presentation by major histocompatibility complex (MHC) class I molecules.[12] The biological function of PA28γ is less well understood and it is unclear whether this complex associates with the 26S proteasome or the 20S proteasome. Nevertheless, PA28γ is thought to play a role in the regulation of transcription or apoptosis.[10]

PA200 is the most recent proteasome activator complex to be discovered and is a single-chain protein of ~200-kDa. It is thought to bind to the ends of the 20S proteasome and may play a role in the repair of DNA damage.[13]

Proteasome activator complexes can also combine with the 20S proteasome to form a variety of hybrid proteasomes. For example, the 20S proteasome may be bound at one end by PA700 and at the opposite end either by PA28 or PA200.[13,14] It has been proposed that within these hybrid proteasomes PA28 and PA200 may function as adapter molecules recruiting proteasomes to specific cellular locations.[10]

The UP pathway has recently attracted much attention in the field of cancer research and proteasome inhibition has been well established as a strategy for the effective management of multiple myeloma[15,16] and non-Hodgkin's lymphoma.[17] This is because proteasome inhibition has been shown to induce the apoptotic cell death of tumour cells.[18] Bortezomib (PS-341; Velcade®) is a dipeptide boronic acid analogue, which has been shown to inhibit the PSMB5 ('chymotrypsin-like' site) and the PSMB1 ('caspase-like' site) subunits of the 20S proteasome. This agent has recently received regulatory approval from the US Food and Drug Administration for the treatment of patients with multiple myeloma or mantle cell lymphoma whose disease has progressed after at least one prior therapy.[19] Bortezomib is currently being investigated for the treatment of other haematological aberrations and solid tumours.[20]

Resistance to conventional anticancer strategies has been long established as a major obstacle in the effective management of human cancers. Various studies have shown that bortezomib may also sensitise tumour cells to the actions of both chemotherapy and radiotherapy[21–24] and is presently being examined in combination with other cytotoxic agents and treatment modalities in tumour cell lines, animal models and multiple clinical trials.[25–27] Initial results appear promising and, in some cases, resistance to anticancer therapy has been overcome.[23,25] Bortezomib in combination with carboplatin elicited an overall response rate of 47% in patients with recurrent ovarian or primary peritoneal cancer, including one complete response in a patient with platinum-resistant disease.[25]

2. The UP pathway and resistance to anticancer therapy

Therapeutic targets upstream of the proteasome may also alter response to anticancer therapy, including members regulating Ub-conjugation to the target substrate. However, conflicting evidence exists as to the roles played by the UP pathway in resistance to anticancer therapy. This review will endeavour to discuss these roles.

2.1. *Enhanced proteasomal activity as a mediator of resistance to therapy*

In 1995, Usui and colleagues cloned a gene named *sks1+* from a gene library of the parental *Schizosaccharomyces pombe* (*S. pombe*) chromosomal DNA.[28] This gene was a suppressor of K-252a-sensitivity when present on a multicopy plasmid. K-252a, an indolocarbazole derivative, is a cytotoxic agent that inhibits a broad spectrum of protein kinases. Parental strains carrying multiple copies of the *sks1+* gene also showed distinct cross-resistance to staurosporine, thiabendazole and vanadate. Nagao and colleagues (1995) isolated a gene called *bfr1+* in a screen for brefeldin-A (BFA)-resistance genes.[29] *Bfr1+* encodes a structural and functional homologue of P-glycoprotein (P-gp) in *S. pombe* cells and was found to be associated with the multi-drug resistance (MDR) phenotype when present on a multicopy plasmid. Further screening for BFA-resistance genes led to the isolation of a gene called *bfr2+*.[30] *Bfr2+* was found to be identical to the *sks1+* gene and a further gene called *pad1+*. The *pad1+* gene was originally isolated as a truncated gene on a multicopy plasmid that was capable of conferring staurosporine-resistance in a wild-type strain of *S. pombe*.[31] Wild-type *S. pombe* cells transformed with a multicopy plasmid containing the *bfr2+/sks1+/pad1+* gene were resistant to staurosporine, K-252a, actinomycin D and cytochalasin B indicating the involvement of this gene in MDR. The MDR conferred by over-expression of the *bfr2+/sks1+/pad1+* gene was found to be dependent on the expression of the *pap1+* gene. The *pap1+* gene product is a *S. pombe* homologue of the mammalian transcriptional activator AP-1. In addition, resistance mediated by *brf2+/sks1+/pad1+* and *pap1* to BFA, K-252a and actinomycin D was also dependent on the expression of *bfr1+*.[30] In the upstream region of the *bfr1+* gene, several repeats of AP-1-like consensus sequences and sequences similar to the palindromic sequence reported to be essential for *pap1+* dependent transcription were found. It was therefore proposed that the over-expression of *bfr2+/sks1+/pad1+* and *pap1+* genes may lead to the transcriptional activation of the *bfr1+* gene leading to MDR. However, it was found that despite the presence of potential binding sites in the 5′ region of the *bfr1+* gene, the level of *bfr1+* transcription did not increase in *S. pombe* cells over-expressing *bfr2+/sks1+/pad1+* or *pap1+* genes. This suggested the indirect regulation of *bfr1+* and it was therefore hypothesised that the *bfr2+/*

sks1+/pad1+ and *pap1+* genes may result in the increased transcription of a gene which augments the drug transport activity of the *bfr1+* gene product possibly via an unknown post-transcriptional modification.[30]

It was discovered that the *bfr2+/sks1+/pad1+* human homologue is *poh1+*. The *poh1+* gene encodes the non-ATPase regulatory subunit 14 of the mammalian 26S proteasome. It is therefore possible that the over-expression of *bfr2+/sks1+/pad1+* may have enhanced the proteasomal degradation of an inhibitor of the *pap1+* gene product allowing the *pap1+* mediated transcription of those genes involved with drug resistance. Alternatively, there is evidence to suggest that components of the 26S proteasome may also serve as transcriptional co-activators. The budding yeast protein, Sug1, and its mammalian counterpart, mSug1/Trip1/FZA-B, have been described both as transcriptional co-activators and subunits of the 26S proteasome.[32–34] Furthermore, mammalian mSug1/Trip1/FZA-B associates with the AP-1 transcription factor c-Fos via its leucine zipper[35] and also with a number of transcription factors from the nuclear receptor family.[36] The over-expression of such components may therefore increase the activation of specific transcription factors. Spataro and colleagues (1997) found that *poh1+* was also able to confer resistance to paclitaxel, doxorubicin, 7-hydroxy-staurosporine and ultraviolet (UV) light when transiently over-expressed in mammalian cells.[37] Further analyses revealed that this resistance was independent of P-gp expression. However, the hypothesis remained that drug resistance induced by *poh1+* may have been mediated through AP-1 transcription factors. Mammalian AP-1 responsive genes include those coding for glutathione-S-transferase Ya and π, elevated levels of which have been associated with cellular resistance to anticancer therapy.[38,39] In addition, the elevated expression of the c-Jun and c-Fos transcription factors has also been correlated with resistance to chemotherapy.[39–41] Thus, it is certainly plausible that the co-ordination of proteolysis with transcriptional activation could be an important parameter in the modulation of transcriptional response to anticancer therapy.

The over-expression of other specific 26S proteasome subunits has also been associated with chemotherapy resistance in mammalian cells. Spataro and colleagues (2002) demonstrated that over-expression of subunit Rpn11 of the PA700 proteasome activator complex was able to confer a moderate level of resistance to vinblastine, cisplatin and doxorubicin in a panel of human cancer cell lines.[42] Furthermore, in a recent study conducted within our laboratory the over-expression of subunit PSMB1 of the 20S proteasome was associated with resistance to cisplatin in MCF7 breast cancer cells.[75] Spataro and colleagues (2002) proposed that under normal physiological conditions proteasome components are co-ordinately expressed under fixed stoichiometric relationships and as such aberrant subunit expression may interfere with proteasome functions resulting in defective protein degradation.[42] It has also been proposed that the over-expression of specific subunits may enhance proteasomal function resulting in the increased degradation of proteins which protect against resistance to anticancer therapy. For example, Chk2 kinase is a protein that becomes activated by the DNA-damage responsive kinase, ATM. Once activated, Chk2 kinase can phosphorylate and activate other downstream targets such as CDC25A, CDC25C, BRCA1, p53 and E2F. These target molecules are important for cell cycle checkpoint regulation, DNA repair and apoptosis. In 2002, Hirao and colleagues showed that Chk2 kinase is critical for DNA damage-induced apoptosis because Chk2 null cells from knockout mice were found to be resistant to ionising radiation.[43] This resistance was attributed to the down-regulation of p53-regulated genes such as Bax and NOXA. Furthermore, the increased proteasomal degradation of Chk2 kinase was found to be responsible for cisplatin-resistance in ovarian cancer cells.[44] Several studies have also revealed pro-apoptotic proteins as substrates for proteasomal degradation. These include p53, Bax, Bik and tBid.[45–48] Specific caspases, which are a family of proteins that mediate the execution of apoptosis, are also substrates for the 26S proteasome.[49,50] This promotes the hypothesis that enhanced proteasomal function may result in an attenuated apoptotic response to anticancer therapy due to the rapid degradation of apoptosis-promoting proteins. In addition, there is evidence to suggest that the 26S proteasome is involved in the regulation of the NF-κB pathway. NF-κB is a transcription factor, which protects cells from apoptosis through the induction of survival genes including cIAP1, cIAP2, XIAP and Bcl-2. In unstimulated cells, an inhibitor of NF-κB (IκB) sequesters NF-κB in the cytoplasm by binding and masking the nuclear translocation signal.[51] In response to a variety of extracellular stimuli, IκB becomes rapidly phosphorylated and ubiquitinylated. Ubiquitinylated IκB is degraded by the 26S proteasome allowing NF-κB to translocate to the nucleus and induce gene transcription. Thus, over-expression of the 26S proteasome may result in the excessive degradation of IκB, which would allow NF-κB to translocate to the nucleus and stimulate the inappropriate transcription of survival genes. In support of this hypothesis Cusack and colleagues (2001) found that administration of bortezomib blocked inducible NF-κB activation, which increased levels of apoptosis in response to chemotherapy.[21] Furthermore, the use of alternative proteasome inhibitors has been reported to increase the efficacy of radiotherapy in human melanoma cells via suppression of the NF-κB pathway.[52] Alternatively, it has been suggested that enhanced proteasomal activity may contribute to resistance to anticancer therapy due to the effective removal of abnormal proteins; the accumulation of which would otherwise induce apoptosis.[53]

Using *Saccharomyces cerevisiae* (*S. cerevisiae*), Jelinsky and colleagues (2000) showed that a large number of protein degradation genes and a selection of nucleotide excision and base excision repair genes are linked in a transcriptional regulon controlled by Rpn4, which is also a proteasome-associated protein.[54] Rpn4 is a transcription factor and stimulates the transcription of these genes in response to a wide range of chemical and physical DNA-damaging agents, including the monofunctional SN2 alkylating agent methyl methanesulphonate (MMS). The *MAG1* gene encodes a 3-methyladenine DNA glycosylase, which is important for base excision repair (BER) and alkylation-resistance. Jelinsky and colleagues demonstrated that the largest category of genes co-regulated with *MAG1* in response to MMS treatment were those involved with protein degradation and suggested that exposure to such agents may signal the induction of a programme to

eliminate and replace alkylated proteins.[54] The Rpn4 protein activates transcription by binding to damage repair consensus elements in the upstream regions of co-regulated genes. In *rpn4* deletion strains of *S. cerevisiae* a dramatic loss of *MAG1* MMS-inducibility was observed. The loss of inducibility of the *RAD23* nucleotide excision repair (NER) gene and the *PRE2* proteasome subunit gene was also observed.[54] Thus, Rpn4 was found to influence the regulation of genes involved in at least three different pathways: BER, NER, and protein degradation. *RAD23* and *SSL2* are NER genes, which were co-regulated with *MAG1* and protein degradation genes. The Rad23 protein has an N-terminal ubiquitin-like domain that interacts with the 26S proteasome and a C-terminus that interacts with another DNA repair protein called Rad4. The Rad23–Rad4 complex interacts with TFIIH (that contains the Ssl2 protein), which is a transcription factor known to be required for NER. Thus, the over-expression of 26S proteasome genes may result in enhanced NER and BER activity, which may reduce the efficacy of anticancer therapy. This may also help to explain why the use of proteasome inhibitors can sensitise tumour cells to the therapeutic effects of ionising radiation.

The over-expression of other members of the UP pathway has also been implicated with resistance to anticancer therapy. Our own unpublished findings suggest that the over-expression of Ub-activating (E1) enzyme may be associated with resistance to radiotherapy in T47D human breast cancer cells. This may result from the inappropriate targeting of apoptosis-promoting proteins for proteasomal degradation thereby reducing cellular response to anticancer therapy. Alternatively, there is evidence to suggest that the Ub-conjugation system may play a direct role in the repair of DNA damage in mammalian cells. Ikehata and colleagues (1997) demonstrated that temperature-sensitive E1 mutants of mouse FM3A cells were defective in UV resistance and induced mutagenicity.[55] This phenotype was found to be reminiscent of a *rad6* DNA repair mutant of *S. cerevisiae* which was previously found to be defective in the Ub-conjugating (E2) enzyme UBC2.[56] In support of this hypothesis, elevated levels of the Ub-conjugating (E2) enzyme CDC34 have been implicated with the growth and survival of multiple myeloma cells. The inhibition of this E2 enzyme was also shown to enhance the anticancer activity of bortezomib, dexamethasone and 2-methoxyestradiol.[57]

However, the most paradigmatic example of how the Ub-conjugation system may contribute to resistance to anticancer therapy is perhaps provided by the Mdm2 protein, a member of the RING-H2 finger super-family of Ub-ligating (E3) enzymes. This protein acts to regulate the function of the p53 tumour suppressor protein, a protein which is frequently mutated in human cancers and has been previously associated with resistance to anticancer therapy. Mdm2 is capable of targeting both itself and p53 for proteasomal degradation. The balance between self or target ubiquitinylation depends largely on post-translational modifications, such as sumoylation and phosphorylation. Under normal conditions, the sumoylation of Mdm2 shifts the E3 ligase activity towards the p53 protein minimising self-ubiquitinylation and promoting the nuclear export and proteasomal degradation of this important regulatory protein. In response to DNA damage, the level of Mdm2 sumoylation is reduced resulting in an increase of self-ubiquitinylation and proteasomal degradation and the stabilisation of p53. In addition, the p53 protein also becomes phosphorylated at many sites, including the Mdm2 binding site, abolishing the formation of Mdm2-p53 complexes and allowing the p53-mediated trans-activation of DNA repair and/or apoptotic genes. The over-expression of Mdm2 has been associated with resistance to anticancer therapy possibly via the inappropriate targeting of p53 for proteasomal degradation in wild-type cells thereby reducing p53-dependent apoptosis in response to treatment.[58,59] Mdm2 is therefore an attractive therapeutic target and silencing strategies have been shown to be effective for the *in vitro* and *in vivo* sensitisation of human cancer cells to chemotherapy and radiotherapy.[60–62]

The stability of the p53 protein is also regulated by the rate of de-ubiquitinylation. HAUSP ubiquitin hydrolase is a DUB enzyme, which removes the Ub moieties from Ub-modified p53 resulting in the stabilisation of this important regulatory protein.[63] It is therefore conceivable that the decreased expression of HAUSP may increase the rate of p53 degradation leading to an attenuated p53-dependent response to anticancer therapy. Li and colleagues (2004) demonstrated that a partial reduction of endogenous HAUSP levels by RNA interference did indeed lead to the destabilisation of p53.[63] HAUSP has also been shown to regulate the activity of the Mdm2 protein. HAUSP de-ubiquitinylates and stabilises the Mdm2 protein thereby resulting in an increased degradation of the p53 protein. Thus, the decreased expression of HAUSP may increase the rate of Mdm2 degradation (rather than p53 degradation) therefore enhancing (rather than attenuating) cellular response to anticancer therapy. In accordance, the complete ablation of HAUSP was found to destabilise Mdm2 leading to the indirect activation of p53 function.[63] These findings suggest an interesting role for HAUSP in the regulation of the p53 pathway and alterations in the expression of this protein may alter cellular response to anticancer therapy.

2.2. *Reduced proteasomal activity as a mediator of resistance to therapy*

In 1988, McCusker and Haber generated a series of temperature-sensitive lethal mutants in *S. cerevisiae* which were resistant to the minimum inhibitory concentration of the protein synthesis inhibitor, cyclohexamide.[64] Gerlinger and colleagues (1997) showed that the *crl* (cyclohexamide-resistant ts lethal)3–2 mutant had a mutation within the *CRL3* gene. *CRL3* was found to be allelic with the *SUG1/CIM3* gene, which encodes for a subunit of the PA700 (19S regulatory) proteasome activator complex of the 26S proteasome.[65] Furthermore, another *crl* mutation *crl21* was allelic with the 20S proteasome β-type gene *PRE3+*. These mutants were defective in proteasomal degradation and demonstrated the characteristic intracellular accumulation of ubiquitinylated proteins. It was also shown that other mutations in various genes encoding subunits of the 20S proteasome subunits (*pre1*, *pre2*, *pre3*, *pre4*) reduced proteolytic activity and also caused resistance to cyclohexamide. Likewise, genes encoding subunits of the 26S proteasome were found closely

linked to 9 of 12 chromosomal positions to which the other *crl* mutations were mapped. It was therefore concluded that the cyclohexamide-resistant phenotypes of most, if not all of the *crl* mutants, were the result of defects in proteasomal degradation.

A screen to isolate mutants that were both resistant to the microtubule-destabilising drug methyl-benzimidazol-2-yl-carbamate (MBC) and temperature-sensitive for growth in *S. pombe* also isolated a series of conditional lethal mutants (*mts2*, *mts3*, *mts4*, and *mts5*) with defects in specific subunits of the 26S proteasome.[66,67] All proteasome mutants showed reduced levels of ubiquitin-conjugated protein degradation indicating defective proteasomal activity. The predicted amino-acid sequence of the cloned *mts2+* gene product was found to be 75% identical to subunit 4 (an ATPase subunit) of the mammalian 26S proteasome. In addition, the deduced amino-acid sequence for the *mts3+* gene product had a high degree of similarity to some of the peptide fragments obtained from subunit 14 (a non-ATPase subunit) of the mammalian 26S proteasome. The size of the *mts3+* gene product is also similar to that found for mammalian subunit 14[68] and is present in the 26S proteasome affinity-purified from fission yeast.[69] The *mts4+* gene encodes a protein with 48% sequence homology to subunit S2 (a non-ATPase subunit) of the mammalian 26S proteasome. Furthermore, the *mts5* mutant was found to harbour a mutation in the *pad1+* gene.[31,67] As described earlier, *pad1+* was originally isolated as a truncated gene on a multicopy plasmid which conferred staurosporine-resistance in wild-type *S. pombe* cells. Further analysis showed that this *pad1+* gene produced a truncated protein product, which lacked 29 amino-acids from the C-terminus.[31,67] An increase in the expression of this mutated gene was found to stimulate *pap1+* (AP1 homologue) dependent transcription. Penney and colleagues (1998) hypothesised that the mutated *pad1+* gene produced an abnormal protein, which disrupted the function of the 26S proteasome leading to staurosporine-resistance possibly due to the decreased proteolysis of a protein required for resistance to this drug.[67]

Recent evidence has also established a link between proteasomal activity and the expression of P-gp in mammalian cells. In 2001, Xu and colleagues devised an approach for the intracellular expression of random peptide libraries in mammalian cells and selected peptides that inhibited taxol-mediated cell death in HeLa cells.[70] It was found that taxol-induced cell death was inhibited when the RGP8.5 peptide was expressed. The RGP8.5 peptide was found to up-regulate the expression of the *ABCB1* (P-gp) gene. Further analyses revealed that this peptide associated with the α-type proteasome subunits PSMA7 and PSMA5 and regulated the expression of the *ABCB1* gene by inhibiting proteasome-mediated protein degradation. Thus, it was proposed that defective proteasomal degradation may lead to the stabilisation of transcription factors which may in turn increase the transcription of those genes required for drug-resistance (e.g. *ABCB1*). In support of this hypothesis, the expression of heat shock proteins is also regulated by the 26S proteasome. Kawazoe and colleagues (1998) showed that heat shock inducible factors were activated by inhibition of the 26S proteasome[71] and such heat shock factors are involved in regulating the transcription of the *MDR1* gene.[72]

Our own unpublished findings also suggest that defects in proteasomal degradation may contribute to resistance to anticancer therapy. Recent results from our laboratory have shown that the down-regulation of specific proteasome-associated subunits may play a role in resistance to radiotherapy (RT) in human breast cancer cells. Several novel derivative cell lines demonstrating significant resistance to ionising radiation were established from breast cancer cell lines. A complementary screening approach comprising two-dimensional gel electrophoresis in combination with matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry and gene expression microarrays revealed the down-regulation of several proteasome subunits in one or more of the RT resistant derivatives. In addition, the up-regulation of a DUB enzyme was also found to be associated with RT resistance in these cells. DUB enzymes act to cleave Ub from target substrates thereby preventing proteasomal degradation. The mechanism(s) by which such alterations may contribute to RT resistance is currently under investigation within our laboratory, however, several possible hypotheses have been proposed. Key cell-cycle regulators (e.g. cyclins and cyclin-dependent kinase inhibitors) are major substrates for proteasomal degradation. Thus, it is possible that the inefficient degradation of those proteins may prevent cell cycle arrest in response to RT-induced DNA damage. The turnover of anti-apoptotic proteins such as Mcl-1 and Bfl-1/A1 is also under the influence of the 26S proteasome.[73,74] Thus, defective proteasomal degradation may result in the inappropriate stabilisation of those proteins harbouring cell survival properties. Furthermore, RT has been shown to induce the proliferation of tumour cells to accelerate re-population following treatment with ionising radiation. This involves the activation of specific cell signalling pathways, including the EGFR and PI3-K pathways. Defects in the 26S proteasome may prevent the effective degradation of key signalling proteins leading to the sustained activation of these cell proliferation pathways.

3. Conclusions

In conclusion, the UP pathway plays a significant role in the regulation of many important biological functions. It is not surprising, therefore, that alterations within this pathway have been shown to contribute to the pathology of many human diseases, including cancer. Proteasome inhibition has been well established as a rational strategy for the treatment of multiple myeloma patients and is currently being investigated for the treatment of other haematological malignancies and solid tumours. Compelling evidence exists to suggest that proteasome inhibition may also sensitise tumour cells to the actions of conventional anticancer strategies. However, questions still remain regarding the roles of this pathway in resistance to anticancer therapy. Many studies have implicated the over-expression of several proteins involved in this pathway with resistance to anticancer therapy and have suggested enhanced proteasomal activity as a mediator of this resistance. However, there is some evidence that the down-regulation of proteasome subunits may also

lead to therapy resistance. Further elucidation of the UP pathway is therefore required in order to fully understand its biological functions and to determine the roles of this pathway in resistance to anticancer therapy. Ultimately, this may aid the identification of novel targets for future therapeutic intervention.

Conflict of interest statement

None declared.

REFERENCES

1. Dalton WS. The proteasome. *Semin Oncol* 2004;**31**:3–9.
2. Kuhlbrodt K, Mouysset J, Hoppe T. Orchestra for assembly and fate of polyubiquitin chains. *Essays Biochem* 2005;**41**:1–14.
3. Pickart CM. Mechanisms underlying ubiquitination. *Annu Rev Biochem* 2001;**70**:503–33.
4. Nijman SM, Luna-Vargas MP, Velds A, et al. A genomic and functional inventory of deubiquitinating enzymes. *Cell* 2005;**123**:773–86.
5. Hoppe T. Multiubiquitylation by E4 enzymes: 'one size' doesn't fit all. *Trends Biochem Sci* 2005;**30**:183–7.
6. Hochrainer K, Lipp J. Ubiquitylation within signaling pathways in- and outside of inflammation. *Thromb Haemost* 2007;**97**:370–7.
7. Wang X, Chen CF, Baker PR, Chen PL, Kaiser P, Huang L. Mass spectrometric characterization of the affinity-purified human 26S proteasome complex. *Biochemistry* 2007;**46**:3553–65.
8. Hirano Y, Hayashi H, Iemura S, et al. Cooperation of multiple chaperones required for the assembly of mammalian 20S proteasomes. *Mol Cell* 2006;**24**:977–84.
9. Groll M, Bajorek M, Kohler A, et al. A gated channel into the proteasome core particle. *Nat Struct Biol* 2000;**7**:1062–7.
10. Rechsteiner M, Hill CP. Mobilizing the proteolytic machine: cell biological roles of proteasome activators and inhibitors. *Trends Cell Biol* 2005;**15**:27–33.
11. DeMartino GN. Purification of PA700, the 19S regulatory complex of the 26S proteasome. *Methods Enzymol* 2005;**398**:295–306.
12. Khan S, van den Broek M, Schwarz K, de Giuli R, Diener PA, Groettrup M. Immunoproteasomes largely replace constitutive proteasomes during an antiviral and antibacterial immune response in the liver. *J Immunol* 2001;**167**:6859–68.
13. Ustrell V, Hoffmann L, Pratt G, Rechsteiner M. PA200, a nuclear proteasome activator involved in DNA repair. *EMBO J* 2003;**21**:3516–25.
14. Tanahashi N, Murakami Y, Minami Y, Shimbara N, Hendil KB, Tanaka K. Hybrid proteasomes: induction by interferon-γ and contribution to ATP-dependent proteolysis. *J Biol Chem* 2000;**275**:14336–45.
15. Catley L, Tai YT, Chauhan D, Anderson KC. Perspectives for combination therapy to overcome drug-resistant multiple myeloma. *Drug Res Updates* 2005;**8**:205–18.
16. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med* 2005;**352**:2487–98.
17. Goy A, Younes A, McLaughlin P, et al. Phase II study of proteasome inhibitor bortezomib in relapsed or refractory B-cell non-Hodgkin's lymphoma. *J Clin Oncol* 2005;**23**:667–75.
18. Demarchi F, Brancolini C. Altering protein turnover in tumor cells: new opportunities for anti-cancer therapies. *Drug Res Updates* 2005;**8**:359–68.
19. Bogner C, Peschel C, Decker T. Targeting the proteasome in mantle cell lymphoma: a promising therapeutic approach. *Leuk Lymphoma* 2006;**47**:195–205.
20. Milano A, Iaffaioli RV, Caponigro F. The proteasome: a worthwhile target for the treatment of solid tumours? *Eur J Cancer* 2007;**43**:1125–33.
21. Cusack JC, Liu R, Houston M, et al. Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: implications for systemic nuclear factor-kappaB inhibition. *Cancer Res* 2001;**61**:3535–40.
22. Russo SM, Tepper JE, Baldwin AS, et al. Enhancement of radiosensitivity by proteasome inhibition: implications for a role of NF-κB. *Int J Radiat Oncol Biol Phys* 2001;**50**:183–93.
23. Ma MH, Yang HH, Parker K, et al. The proteasome inhibitor PS-341 markedly enhances sensitivity of multiple myeloma tumor cells to chemotherapeutic agents. *Clin Cancer Res* 2003;**9**:1136–44.
24. Mitsiades N, Mitsiades CS, Richardson PG, et al. The proteasome inhibitor PS-341 potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: therapeutic applications. *Blood* 2003;**101**:2377–80.
25. Aghajanian C, Dizon DS, Sabbatini P, Raizer JJ, Dupont J, Spriggs DR. Phase I trial of bortezomib and carboplatin in recurrent ovarian or primary peritoneal cancer. *J Clin Oncol* 2005;**23**:5943–9.
26. Orlowski RZ, Voorhees PM, Garcia RA, et al. Phase I trial of the proteasome inhibitor bortezomib and pegylated liposomal doxorubicin in patients with advanced hematologic malignancies. *Blood* 2005;**105**:3058–65.
27. Jagannath S, Richardson PG, Barlogie B, et al. Bortezomib in combination with dexamethasone for the treatment of patients with relapsed and/or refractory multiple myeloma with less than optimal response to bortezomib alone. *Haematologica* 2006;**91**:929–34.
28. Usui T, Yoshida M, Honda A, Beppu T, Horinouchi S. A K-252a-resistance gene, *sks1+*, encodes a protein similar to the *Caenorhabditis elegans F37 A45* gene product and confers multidrug resistance in *Schizosaccharomyces pombe*. *Gene* 1995;**161**:93–6.
29. Nagao K, Taguchi Y, Arioka M, et al. *bfr1+*, a novel gene of *Schizosaccharomyces pombe* which confers brefeldin A resistance, is structurally related to the ATP-binding cassette superfamily. *J Bacteriol* 1995;**177**:1536–43.
30. Arioka M, Kouhashi M, Yoda K, Takatsuki A, Yamasaki M, Kitamoto K. Multidrug resistance phenotype conferred by over-expressing *bfr2+/pad1+/sks1+* or *pap1+* genes and mediated by *bfr1+* gene product, a structural and functional homologue of P-glycoprotein in *Schizosaccharomyces pombe*. *Biosci Biotechnol Biochem* 1998;**62**:390–2.
31. Shimanuki M, Saka Y, Yanagida M, Toda T. A novel essential fission yeast gene *pad1+* positively regulates *pap1+*-dependent transcription and is implicated in the maintenance of chromosome structure. *J Cell Sci* 1995;**108**:569–79.
32. Swaffield JC, Bromberg JF, Johnston SA. Alterations in a yeast protein resembling HIV Tat-binding protein relieve requirement for acidic activation domain in GAL4. *Nature* 1992;**357**:698–700.
33. Akiyama K, Yokota K, Kagawa S, et al. cDNA cloning of a new putative ATPase subunit p45 of the human 26S proteasome, a homolog of yeast transcriptional factor Sug1p. *FEBS Lett* 1995;**363**:151–6.
34. Rubin DM, Coux O, Wefes I, et al. Identification of the gal4 suppressor Sug1 as a subunit of the yeast 26S proteasome. *Nature* 1996;**379**:655–7.

35. Wang W, Chevray PM, Nathans D. Mammalian Sug1 and c-Fos in the nuclear 26S proteasome. *Proc Natl Acad Sci USA* 1996;**93**:8236–40.
36. vom Baur E, Zechel C, Heery D, et al. Differential ligand-dependent interactions between the AF-2 activating domain of nuclear receptors and the putative transcriptional intermediary factors mSUG1 and TIF1. *EMBO J* 1996;**15**:110–24.
37. Spataro V, Toda T, Craig R, et al. Resistance to diverse drugs and ultraviolet light conferred by overexpression of a novel human 26S proteasome subunit. *J Biol Chem* 1997;**272**:30470–5.
38. Pinkus R, Bergelson S, Danial V. Phenobarbital induction of AP-1 binding activity mediates activation of glutathione *S*-transferase and quinine reductase gene expression. *Biochem J* 1993;**290**:637–40.
39. Moffat GJ, McLaren AW, Wold CR. Involvement of Jun and Fos proteins in regulating transcriptional activation of the human pi class glutathione *S*-transferase gene in multidrug-resistant MCF7 breast cancer cells. *J Biol Chem* 1994;**269**:16397–402.
40. Ritke MK, Bergoltz VV, Allan WP, Yalowich JC. Increased c-jun/AP-1 levels in etoposide-resistant human leukaemia K562 cells. *Biochem Pharmacol* 1994;**48**:525–33.
41. Yao KS, Godwin AK, Johnson SW, Ozols RF, O'Dwyer PJ, Hamilton TC. Evidence for altered regulation of γ-glutamylcysteine synthetase gene expression among cisplatin-sensitive and cisplatin-resistant human ovarian cancer cell lines. *Cancer Res* 1995;**55**:4367–74.
42. Spataro V, Simmen K, Realini CA. The essential 26S proteasome subunit Rpn11 confers multidrug resistance to mammalian cells. *Anticancer Res* 2002;**22**:3905–10.
43. Hirao A, Cheung A, Duncan G, et al. Chk2 is a tumour suppressor that regulates apoptosis in both an ataxia telangiectasia mutated (ATM)-dependent and an ATM-independent manner. *Mol Cell Biol* 2002;**22**:6521–32.
44. Zhang P, Gao W, Li H, Reed E, Chen F. Inducible degradation of checkpoint kinase 2 links to cisplatin-induced resistance in ovarian cancer cells. *Biochem Biophys Res Commun* 2005;**328**:567–72.
45. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature* 1997;**387**:296–9.
46. Li B, Dou QP. Bax degradation by the ubiquitin/proteasome pathway: involvement in tumour survival and progression. *Proc Natl Acad Sci USA* 2000;**97**:3850–5.
47. Marshansky V, Wang X, Bertrand R, et al. Proteasomes modulate balance among pro-apoptotic and anti apoptotic Bcl-2 family members and compromise functioning of the electron transport chain in leukemic cells. *J Immunol* 2001;**166**:3130–42.
48. Breitschopf K, Zeiher AM, Dimmeler S. Ubiquitin-mediated degradation of the proapoptotic active form of bid. A functional consequence on apoptosis induction. *J Biol Chem* 2000;**275**:21648–52.
49. Huang H, Joazeiro CA, Bonfocco E, Kamada S, Leverson JD, Hunter T. The inhibitor of apoptosis, cIAP2, functions as a ubiquitin-protein ligase and promotes in vitro monoubiquitination of caspases 3 and 7. *J Biol Chem* 2000;**275**:26661–4.
50. Suzuki Y, Nakabayashi Y, Takahashi R. Ubiquitin-protein ligase activity of x-linked inhibitor of apoptosis protein promotes proteasomal degradation of caspase-3 and enhances its anti-apoptotic effect if Fas-induced cell death. *Proc Natl Acad Sci USA* 2001;**98**:8662–7.
51. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol* 2000;**18**:621–63.
52. Munshi A, Kurland JF, Nishikawa T, Chiao PJ, Andreeff M, Meyn RE. Inhibition of constitutively activated nuclear factor-κB radiosensitizes human melanoma cells. *Mol Cancer Ther* 2004;**3**:985–92.
53. Taylor RC, Adrian C, Martin SJ. Proteases, proteasomes and apoptosis: breaking Ub is hard to do. *Cell Death Differ* 2005;**12**:1213–7.
54. Jelinsky SA, Estep P, Church GM, Samson LD. Regulatory networks revealed by transcriptional profiling of damaged *Saccharomyces cerevisiae* cells: Rpn4 links with base excision repair with proteasomes. *Mol Cell Biol* 2000;**20**:8157–67.
55. Ikehata H, Kaneda S, Yamao F, Seno T, Ono T, Hanaoka F. Incubation at the nonpermissive temperature induces deficiencies in UV resistance and mutagenesis in mouse mutant cells expressing a temperature-sensitive ubiquitin-activating enzyme (E1). *Mol Cell Biol* 1997;**17**:1484–9.
56. Jentsch S, McGrath JP, Varshavsky A. The yeast DNA repair gene RAD6 encodes a ubiquitin-conjugating enzyme. *Nature* 1987;**329**:131–4.
57. Chauhan D, Li G, Hideshima T, et al. Blockade of the ubiquitin-conjugating enzyme CDC34 enhances anti myeloma activity of Bortezomib/Proteasome inhibitor PS-341. *Oncogene* 2004;**23**:3597–602.
58. Suzuki A, Toi M, Yamamoto Y, Saji S, Muta M, Tominaga T. Role of MDM2 overexpression in doxorubicin resistance of breast carcinoma. *Jpn J Cancer Res* 1998;**89**:221–7.
59. Sjostrom J, Blomqvist C, Heikkila P, et al. Predictive value of p53, mdm-2, p21 and mib-1 for chemotherapy response in advanced breast cancer. *Clin Cancer Res* 2000;**6**:3103–10.
60. Wang H, Oliver P, Zhang Z, Agrawal S, Zhang R. Chemosensitization and radiosensitization of human cancer by antisense anti-MDM2 oligonucleotides: in vitro and in vivo activities and mechanisms. *Ann N Y Acad Sci* 2003;**1002**:217–35.
61. Kojima K, Konopleva M, McQueen T, O'Brien S, Plunkett W, Andreeff M. Mdm2 inhibitor Nutlin-3a induces p53-mediated apoptosis by transcription-dependent and transcription-independent mechanisms and may overcome Atm-mediated resistance to fludarabine in chronic lymphocytic leukaemia. *Blood* 2006;**108**:993–1000.
62. Yu Y, Sun P, Sun LC, et al. Downregulation of MDM2 expression by RNAi inhibits LoVo human colorectal adenocarcinoma cells growth and the treatment of LoVo cells with mdm2siRNA3 enhances sensitivity to cisplatin. *Biochem Biophys Res Commun* 2006;**339**:71–8.
63. Li M, Brooks CL, Kon N, Gu W. A dynamic role of HAUSP in the p53-Mdm2 pathway. *Mol Cell* 2004;**13**:879–86.
64. McCusker JH, Haber JE. Cycloheximide-resistant temperature-sensitive lethal mutations of *Saccharomyces cerevisiae*. *Genetics* 1988;**119**:305–15.
65. Gerlinger UM, Guckel R, Hoffmann M, Wolf DH, Hilt W. Yeast cycloheximide-resistant *crl* mutants are proteasome mutants defective in protein degradation. *Mol Biol Cell* 1997;**8**:2487–99.
66. Gordon C, McGurk G, Dillon P, Rosen C, Hastie ND. Defective mitosis due to a mutation in the gene for a fission yeast 26S protease subunit. *Nature* 1993;**366**:355–7.
67. Penney M, Wilkinson C, Wallace M, et al. The *pad1+* gene encodes a subunit of the 26S proteasome in fission yeast. *J Biol Chem* 1998;**272**:23938–45.
68. Dubiel W, Ferrell K, Rechsteiner M. Subunits of the regulatory complex of the 26S protease. *Mol Biol Rep* 1995;**21**:27–34.
69. Goldberg AL, Rock KL. Proteolysis, proteasomes and antigen presentation. *Nature* 1992;**357**:375–9.
70. Xu X, Leo C, Jang Y, et al. Dominant effector genetics in mammalian cells. *Nat Genet* 2001;**27**:23–9.
71. Kawazoe Y, Nakai A, Tanabe M, Nagata K. Proteasome inhibition leads to the activation of all members of the heat shock factor family. *Eur J Biochem* 1998;**255**:356–62.

72. Kim SH, Hur WY, Kang CD, Lim YS, Kim DW, Chung BS. Involvement of heat shock factor in regulating transcriptional activation of MDR1 gene in multidrug resistant cells. *Cancer Lett* 1997;**115**:9–14.
73. Zhong Q, Gao W, Du F, Wang X. Mule/ARF-BP1, a BH3-only E3 ubiquitin ligase, catalyzes the polyubiquitination of Mcl-1 and regulates apoptosis. *Cell* 2005;**121**: 1085–95.
74. Kucharczak JF, Simmons MJ, Duckett CS, Gelinas C. Constitutive proteasome-mediated turnover of Bfl-1/A1 and its processing in response to TNF receptor activation in FL512 pro-B cells convert it into a prodeath factor. *Cell Death Differ* 2005;**12**:1225–39.
75. Smith L, Welham KJ, Watson MB, Drew PJ, Lind MJ, Cawkwell L. The proteomic analysis of cisplatin resistance in breast cancer cells. *Oncol Res*, in press.