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Position Paper

Targeting ubiquitin in cancers ☆

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ABSTRACT

Ubiquitin (Ub) is a small protein modifier involved in cellular functions such as cell cycle, apoptosis, cell signalling, endocytosis, transcription and DNA repair. Ubiquitin operates as a reversible and highly versatile regulatory signal, which may be read and interpreted by an expanding number of Ub-binding domains (UBD). There is accumulating evidence that mutations or altered expression of ubiquitylating or de-ubiquitylating enzymes as well as of Ub-binding proteins affect crucial mediators of such functions and are found in several malignancies. Here we discuss how oncogenic alterations in the Ub system can be targeted by anti-cancer therapies.

1. Introduction

Ubiquitin (Ub) has emerged as a versatile molecule, which plays a pivotal role not only in protein degradation, but also in non-proteolytic processes (membrane transport, chromatin structure and transcription, DNA repair and signalling pathways).[1,2] Ubiquitin is conjugated to a target protein via a multi-step process known as ubiquitination or ubiquitylation, which involves an E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzymes and E3 ubiquitin ligases. These last are the 'brains' of the ubiquitination process since they warrant substrate specificity.[1,3] Ubiquitin can be viewed as a sophisticated version of a phosphoryl group that similarly can be attached in a reversible manner to a wide spectrum of proteins. Ubiquitylation is even more versatile since different lysines in a given protein may be tagged by ubiquitin, and Ub itself can undergo ubiquitylation to form polyubiquitin chains of various length and type (Fig. 1). Lastly, Ub can be removed from its targets in a regulated fashion, by the action of one of the increasingly larger group of de-ubiquitylating enzymes (DUB).[1,3] Chains of at least four ubiquitin moieties linked via their lysine 48 represent a specific signature that targets a wide variety of cytosolic and nucleoplasmic proteins for proteasomal degradation. In this proteolytic process, polyubiquitylated substrates are carried by different ubiquitin receptors to the proteasome, where ubiquitins are recycled via ubiquitin C-terminal hydrolases, while they get cleaved into small peptides by proteasomic proteases.[4,5] By governing proteolysis, Ub indirectly controls many cellular functions such as cell cycle progression, differentiation and development, cellular responses to extra-cellular effectors and stress, induction of inflammatory and immune responses and repair or tolerance of DNA damage.

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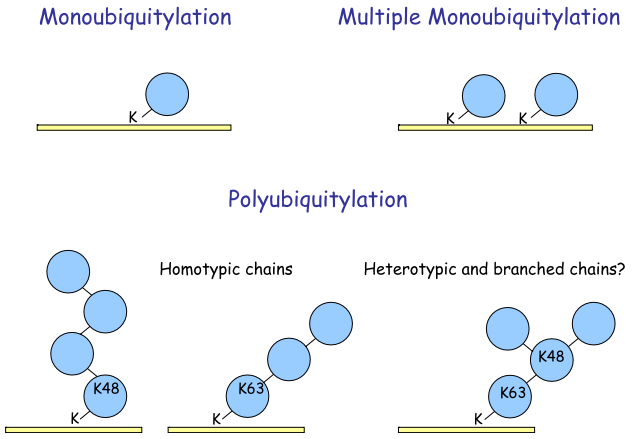

Fig. 1 – Different types of protein modifications by ubiquitin. A single Ub moiety can be covalently attached to one or more lysine residues of a given protein, in a reaction called mono- or multiple mono-ubiquitylation. Because Ub itself bears seven lysine residues, it can serve as a target for other ubiquitins to be attached and form homotypical chains, in a reaction known as polyubiquitylation. Here are depicted the two best characterised types of Ub chains, linked via lysine residues 48 and 63 of Ub. Heterotypic and branched chains may also exist in the cell, although the proof for this is still missing. Yellow bars represent the primary structure of a target protein.

This diversity of reversible modifications and broad range of specificities makes the same signal, ubiquitin, able to convey different information, at different locations in the cell at the same time. At least 80% of cellular proteins (including cell cycle regulators, tumour suppressors, growth modulators, transcriptional activators and inhibitors, cell surface receptors and endoplasmatic reticulum proteins) are targeted by ubiquitin, therefore making such a relatively small oligopeptide of 76 amino acids a master regulator of cellular homeostasis. Interestingly, ubiquitylation of many proteins does not mediate their degradation but rather leads to conformational changes, regulates their subcellular localisation or catalytic activity or engages them in protein–protein interactions with effectors containing small Ub-binding domains (UBD). This leads to the formation of Ub signalling networks, which translate signals from the cellular environment into proper cellular phenotypes (Fig. 2).[2,6]

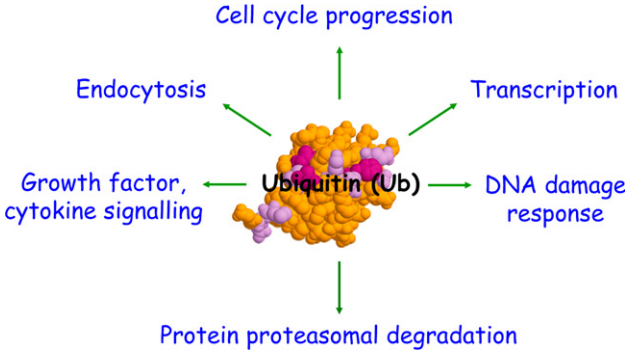

Fig. 2 – Ubiquitin is a master regulator of many cellular functions. This small 76 amino acid polypeptide can be reversibly attached to a huge variety of intracellular proteins. Such modification is sensed, read and interpreted by a variety of non-covalently binding domains harboured by a plethora of proteins with regulatory, adaptory or enzymatic function. The result is a fine regulation of protein turnover as well as cell cycle, endocytosis, gene transcription and signalling, for instance in the TNFα-NFκB pathway.

The first discovery of protein domains able to recognise ubiquitin, UIM and UBA, came after the role of ubiquitin in proteasomal degradation was established. Since then, the family of UBDs has rapidly expanded and it now contains more than 15 annotated members, including UBA, UIM, CUE, UEV, GAT, GLUE, NZF, VHS, PAZ, UBM and UBZ.[7,8] Surprisingly, even though their structures differ quite a lot from each other, almost all of them recognise the same hydrophobic surface on ubiquitin, with isoleucine 44 (Ile44) being crucial. The affinity of the interaction between Ub and UBDs falls in the order of 100 micromolar or less and is usually higher for poly-Ub chains than for mono-Ub. Moreover, each type of domain has a broad range of binding affinities for Ub as measured by experiments with purified domains from different proteins.[7] However, *in vivo* the affinity of UBD-containing proteins for their targets may be enhanced by additional binding sites beside the Ub-UBD interface. These further contacts endow the interaction with specificity, while Ub behaves as an on-off switch that rather tells whether or not the interaction can occur.[9]

Because many of the functions regulated by ubiquitin are often deregulated in human cancers,[10] it is not surprising that the ubiquitin-proteasome pathway along with molecules either involved in the cross-roads of the major signalling cascades or acting as chaperones for key molecules have become targets for novel and more selective anti-cancer treatments.[11,12] In addition, a considerable amount of work in the recent years has provided evidence that mutations or altered expression of ubiquitylating or de-ubiquitylating enzymes as well as of Ub-binding proteins can be found in several malignancies, therefore making it even more appealing to drug the Ub system.[13] Here we summarise the most compelling examples of aberrations in the ubiquitin-proteasome system and review the major anti-cancer drugs which have reached the bedside and target the ubiquitin system in human malignancies.

2. Ubiquitin signalling in tumour cells

Cell cycle disregulation is a universal property of cancers. Transitions through the various phases of the cell cycle are driven by cyclin-dependent kinases (CDKs), which are activated in a time-regulated fashion by periodical oscillation of the concentration of their regulatory subunits, cyclins. [14] Several ubiquitin ligases are crucial in this game: SCF/Skp2 (Skp1-cul1-F-box protein), containing the Skp2 F-box protein, binds to and polyubiquitylates phosphorylated p27 and possibly cyclin D1, while SCF/Fbw7 targets phosphorylated cyclin E for proteasomal degradation.[15] The Anaphase-Promoting

Complex (APC) is required for polyubiquitylation and degradation of securin and cyclin B, which is indispensable for sister chromatid separation and exit from mitosis respectively.[14] In a number of human cancers, cell cycle disregulation is caused by mutations of APC, SCF/Skp2 or Fbw7. Inactivating mutations of the APC subunits Cdc16 and Cdc23 have been described in colon carcinomas and reduced expression of the regulatory subunit Cdh1 was associated with the progression of a B-cell lymphoma line. In addition to mutations in its subunit, APC may also be blocked by high levels of its inhibitor Emi1, which have been detected in several human tumours. Skp2 was found to be overexpressed in a large number of cancer types, where its levels correlated with a poor prognosis. Additionally, Skp2 alone or in association with oncogenic Ras could induce tumour formation in two mouse models and transformation as assessed by colony formation in soft agar.[14–17]

More examples of cancer-associated mutations or aberrant gene expressions have been found to affect transmembrane receptors and endocytic proteins. Endocytosis is a very evolutionary conserved process by which eucaryotes uptake extracellular material or remove parts of their plasma membrane and transmembrane proteins, in an attempt to balance the addition of newly synthesised ones. Ubiquitylation of various cell surface receptors as well as of many components of the endocytic machinery has been found to regulate virtually all steps of endocytosis, including internalisation at the plasma membrane, trafficking of endosomes and delivery of the endocytic cargo into lysosomes.[18] Altered ubiquitin signalling for sorting transmembrane proteins to lysosomes, especially in the context of constitutively active Receptor Tyrosine Kinases (RTK), such as the Epidermal Growth Factor (EGF) receptor, may contribute to the progression of some neoplasms, if not actually being the initiating event. For example, the Cbl E3 ubiquitin ligase ubiquitylates active EGF receptors and other RTKs, targeting them for endocytosis and lysosomal degradation, a process known as downregulation. Interestingly, loss of Cbl binding is found in several oncogenic variants of EGFR, c-Met, CFS-1R and c-Kit, probably leading to their accumulation at the plasmamembrane and sustained signalling.[19] Moreover, quite a few adaptor proteins required for appropriate receptor downregulation have been found to be associated with several human or experimental malignancies, and the mechanisms by which they seem to participate to oncogenesis have already been extensively described elsewhere.[20]

In addition to the major aberrations described above, for which, though little, evidence exists of a mechanistic role in either oncogenic initiation or progression, many other examples of mutations, including translocations, overexpression and aberrant DNA methylation have been found that involve components of the ubiquitin-proteasome pathway, especially in haematological malignancies. For example, MALT lymphomas are the most common type of human lymphomas arising in extranodal sites. The pathogenesis of these haematological diseases has been associated with at least three independent chromosomal translocations, two of which, t(1;14)(q22;q32) and t(14;18)(q32;q21), result in the upregulation of BCL10 and MALT1, while the third and most common one, t(11;18)(q21;q21), generates a fusion protein between cIAP2 and MALT1. Both BCL10 and MALT1 play a critical role in antigen receptor–mediated lymphocyte proliferation and signalling to NFκB, which controls the expression of genes critical for cell survival, proliferation, and immune responses. In particular, BCL10 and MALT1 contribute together to the polyubiquitination of NEMO, a regulator of the inhibitor of NFκB kinase complex, IκB. It has been recently demonstrated that cIAP2 is an E3 ubiquitin ligase, targeting BCL10 for degradation. This function is abolished in cIAP2-MALT1 chimeric product and in t(11;18) lymphomas leads to enhanced BCL10 stability. The cIAP2-MALT1 fusion protein strongly activates NFκB compared with the normal counterpart, MALT1, suggesting a gain-of-function mutant phenotype for the chimeric products.[21–24]

Table 1 – Inhibitors of the ubiquitin-proteasome pathway and of heat shock proteins currently available at patients' bedside, in the context of clinical trials

Target protein	Inhibitor drug
20 S Proteasome	Bortezomib, Velcade (PS-341) NPI-0052 (salinosporin A)
E3 conjugating enzymes	
hMdm2	Nutlin 3
Chaperonines	Geldanamycin 17-allylamino-17-demethoxygeldanamicin (17-AAG)
HSP 90	Shepherdin

We will now describe the major Ub system-targeting drugs available at the patient's bedside (Table 1) and discuss novel aspects in drug discovery and development in targeting ubiquitin in human cancers.

3. Available drugs targeting the Ub system

3.1. *Bortezomib, Velcade® (PS341)*

Bortezomib, a dipeptide boronate, is a potent and reversible inhibitor of the proteasome. Like other natural and synthetic compounds, especially boronic acid derivatives, it potently binds to the chymotrypsin-like site of the 20S proteasome and dissociates slowly, therefore stably but reversibly inhibiting the proteasome.[25] Pre-clinical studies, performed on multiple myeloma (MM) cell lines and freshly isolated plasma cells, demonstrated that Bortezomib blocks the proliferation and causes the apoptosis of plasma cells independently of their sensitivity to chemotherapy. Peripheral blood cells from healthy donors or normal stromal bone marrow cells are >50 fold less sensitive to Bortezomib-induced apoptosis. This last is a caspase-mediated process occurring independently of p53 status, which expression is also enhanced by Bortezomib. Bortezomib also blocks the proteasomal degradation of IkBα, the negative regulator of nuclear factor κB (NF-κB). This action is probably very important in explaining the activity of Bortezomid in patients refractory to chemotherapy, since NF-κB activation has been shown to play a fundamental role in the development of multiple drug resistance.[25–27] Therefore, through this inhibition and synergistic action with chemotherapy, Bortezomib induces either a better response to chemotherapy or restores chemo-sensitivity.[25,26] The inhibition of NF-κB also reduces the expression of genes coding

Table 2 – Bortezomib in refractory/relapsed MM patients: clinical trials

Regimen	Doses	Total Pts	Response rate
SUMMIT	Bort: 1.3mg/m^2 on days 1, 4, 8, 11 (maximum 8 three-week courses) Dex:20mg on day of and day after each bort course if progressive disease after two courses or SD after four	202	CR+PR+MR = 35% Median time to progression = 7mo. Median duration of response = 12mo. Median overall survival = 16mo.
CREST	Bort: 1.0mg/m^2 on days 1, 4, 8, 11 (maximum 8 three week courses) Dex:20mg on day of and day after each bort course if progressive disease after two courses or SD after four *Versus*	27	CR+PR+MR = 33% (44% if dex added) Median time to progression = 7mo. Median duration of response = 9.5 mo. Median overall survival = 26.7mo.
	Bort:1.3mg/m^2 on days 1, 4, 8, 11 (max 8 three week courses) Dex:20mg on day of and day after each bort course if progressive disease after two courses or SD after four	26	CR+PR+MR = 50% (62% if dex added) Median time to progression = 11mo. Median duration of response = 13.7 mo. Median overall survival = not reached
APEX	Bort: 1.3mg/m^2 on days 1, 4, 8, 11 (8 three week-courses), subsequently Bort: 1.3mg/m^2 on days 1, 8, 15, 22 (3 five-week courses) *Versus*	333	CR+PR = 38% 1-year survival = 80% Median time to progression = 6.2 mo.
	Dex: 40mg on days 1–4, 9–12, 17–20 every five weeks for four courses, subsequently five courses on days 1–4 every four weeks	336	CR+PR = 18% 1-year survival = 66% Median time to progression = 3.5 mo.
VTD	Bort: 1.0–1.3mg/m^2 on days 1, 4, 8, 11 every three weeks Thalidomide: 50–200mg daily at the atrat of the second Bort. course Dex: 20mg on day of and day after bort dose beginning from the fourth bort course if PR was not reached	85	CR+PR+MR = 70%. Median event-free survival = 9mo Median OS = 22mo.
Bort + MEL	Bort:0.7–1.0mg/m^2 on days 1, 4, 8,11 (for a maximum of 8 four-week courses) Mel: 0.025–0.25mg/kg on days 1–4	31	CR+PR+MR = 68% Median progression-free survival = 8mo.
Bort + CTX + Dex	Bort: 1.3mg/m^2 on days 1, 4, 8, 11 (8 three-week courses) subsequently Bort: 1.3mg/m^2 on days 1, 8, 15, 22 (3 five-week courses) CTX: 50mg/day Dex: 20mg on day of and day after each bortezomib cycle	50	CR+PR+MR = 90% Median event-free survival = 12mo.
Bort+ Mel + Pred + Thal	Bort: 1.0–1.6mg/m^2 on days 1, 4, 15, 22 (6 five-weeks courses) Mel: 6mg/m^2 on days 1–5 Pred: 60mg/m^2 on days 1–5 Thal: 100mg/day	30	CR+PR = 69%
Bort + PegLD	Bort: 0.9–1.5mg/m^2 on days 1, 4, 8, 11 every three-weeks PegLD: 30mg/m^2 on day 4	22	CR+PR = 73%
Bort + PegLD + Thal	Bort: 1.3mg/m^2 on days 1, 4, 15, 18 for a maximum of six four-week courses or two courses post CR PegLD: 20mg/m^2 on day 1 and 15 Thal: 200mg/day	21	CR+PR+MR = 57%
Bort + Len	Bort: 1.0–1.3mg/m^2 on days 1, 4, 8, 11 every three weeks Len: 5–15mg on days 1–14 every three weeks Dex: 20mg on day of and day after each bortezomib cycle if progressive disease	24	CR+PR = 52%

Bort = Bortezomib; Dex = Dexamethasone; Mel = Melphalan; Thal = Thalidomide; Dox = Doxorubicine; Pred = Prednisone; Len = Lenalidomide; CR = complete remission; PR = partial remission; MR = minimal response.

for pro-inflammatory (IL-6, TNF-α) and cell adhesion molecules, which are secreted by bone marrow stromal cells, and up-regulates cyclin dependent kinase inhibitors p21 and p27. All these activities lead to enhanced apoptosis.[25]

Several clinical studies established Bortezomib safety and therapeutic effectiveness in patients who have not responded to various previous chemotherapy schedules, which also included bone marrow transplantation. The most relevant phase II/III studies are listed in Table 2. The SUMMIT study[28,29]

Table 3 – Bortezomib in newly diagnosed MM patients: clinical trials

Regimen	Doses	Total Pts	Response rate
Bortezomib	Bort: 1.3mg/m^2 on days 1, 4, 8, 11 every three weeks	66	CR+PR = 38%
Bortezomib + Dex	Bort: 1.3mg/m^2 on days 1, 4, 8, 11 (maximum six three- week courses) Dex: 20mg on day of and day after each bortezomib cycle if <PR after two courses or <CR after four courses	50	CR+PR = 90% ⩾near CR = 20%
Bortezomib + Dex	Bort: 1.3mg/m^2 on days 1, 4, 8, 11 (four three-week courses) Dex: 40mg on day 1–4, 9–12 for the first and second courses and on days 1–4 for the third and fourth courses	48	CR+PR = 67% CR = 17%
PAD	Bort: 1.0mg/m^2 on days 1, 4, 8, 11 (four three-week courses) Dox: 9.0mg/m^2 on days 1–4 Dex: 40mg on days 1–4, 8–11, 15–18 in the first course; on days 1–4 in the second, third and fourth courses	19	CR+PR = 89% CR = 11% Near CR = 5% Very good partial response =33%
VTD	Bort: 1.0–1.9mg/m^2 on days 1, 4, 8, 11 (two or three four-week courses) Thal: 100–200mg daily Dex:20mg/m^2 on days 1–4, 9–12, 17–20	38	CR+PR = 92% CR = 18%
V-MP	Bort: 1.0mg/m^2 (group 1:on days 1, 4, 8, 11, 22,25, 29, 32);escalate to 1.3mg/m^2 (group 2) for a maximum of four six-week courses and five five-week courses Mel: 9.0mg/m^2 on days 1–4 Pred: 60mg/m^2 on days 1–4	60	CR+PR = 88% CR = 32% Near CR = 11%

Bort = Bortezomib; Dex = Dexamethasone; Mel = Melphalan; Thal = Thalidomide; Dox = Doxorubicine; Pred = Prednisone; Len = Lenalidomide; CR = complete remission; PR = partial remission; MR = minimal response.

and the CREST study[30] were carried out in MM patients who had received previous therapies or were refractory to the most recent treatments. Both studies concluded that Bortezomib is an effective savage therapy in refractory MM patients exhibiting an *in vivo* dose-response relationship and a synergy with Dexametasone (Dex), since the combination of Bortezomib and Dex improved response rates. More recently, the APEX (Assessment of Proteasome inhibition for Extending remissions)[31] study randomised refractory MM patients to receive Bortezomib or high-dose Dex. In this study CR+PR were 38% for Bortezomib *versus* 18% for Dex and Bortezomib offered a 78% improvement in median time to progression.

Based on the synergism between Bortezomib and chemotherapeutic agents already demonstrated by pre-clinical studies, additional clinical trials have assessed the therapeutic effectiveness of such associations[32–35] (Table 2). One of the most interesting trials evaluated the therapeutic effectiveness of Bortezomib in combination with Pegylated Liposomal Doxorubicin (PLD). Interestingly, patients resistant to Doxorubicin alone were sensitive to combined treatment. The other drugs used in association with Bortezomib were Melphalan, Thalidomide, low-dose Dex. All these trials were aimed at reducing the Bortezomib dose in order to lower its toxicity and at taking advantage of its synergism with anti-neoplastic drugs. A 50–80% response rate was achieved by these treatment schedules.

Given the therapeutic efficacy and tolerability of Bortezomib in refractory/relapsed MM, several clinical trials have also evaluated this drug as front-line therapy in newly diagnosed patients (Table 3). Jagannath and co-workers were the first to report the results of one such study,[36] where the overall response rate was 90% and a clinical response was obtained in 90% of the patients after six cycles. In subsequent studies, Bortezomib has been successfully associated with Doxorubicin and Dex (PAD), Dex alone, Thalidomide and Dex, Melphalan and Prednisone,[37–40] with similar results (Table 3). In addition, it has been demonstrated that autologous stem cell collection was still feasible in these patients despite treatment with chemotherapeutic combinations aimed at killing the maximum plasma cell number thanks to the synergistic action of Bortezomib with other drugs.

Given its clinical success in multiple myeloma, several clinical trials have started to address the feasability of Bortezomib-containing schedules in other haematological malignancies. In mantle cell lymphomas (MCL), where NF-κB is constitutively active, Bortezomib has been shown to induce tumour cell apoptosis by activating caspase-3 and lowering cyclin D1 expression. Other clinical trials, which have used Bortezomib in other malignant lymphomas, have suggested that response rate is determined by the histological subtype of lymphoma and have stressed the need for additional studies in specific subtypes. One of such studies was carried out in patients with various types of refractory lymphoma, including MCL.[41] MCL patients experienced a 41% response rate, but satisfactory results were also obtained in other lymphoma subtypes. This was underlined by another study, which used the same Bortezomib dose. This trial reported a response rate of 50% in MCL, 78% in follicular lymphoma, 100% in marginal cell lymphoma and 0% in small lymphocytic lymphoma. The National Cancer Institute trial, a phase II trial still ongoing, is comparing Bortezomib alone versus Bortezomib in combination with the EPOCH (Etoposide, Prednisone, Vincristine, Cyclophosphamide and Doxorubicine) schedule.[42] Interim results have fixed Bortezomib maximum

tolerated dose at 1.5mg/m^2 and have reported a higher response rate for patients undergoing combined treatment.

3.2. *NPI-0052 (salinosporin A)*

This novel marine-derived proteasome inhibitor acts on the three enzymatic activities of the 20S proteasome in a way different from that of Bortezomib: NPI-0052 activity is more rapid and irreversible.[43] A recent study reported that brief pulses of NPI-0052 were sufficient to induce a substantial apoptosis of chronic lymphoid leukemia (CLL) cells and showed that caspase-4 mediated apoptosis was enhanced in comparison to that induced by Bortezomib.[44] Another pre-clinical study, carried out on MM cells resistant to conventional chemotherapy and Bortezomib, suggested that NPI-0052 is distinct from Bortezomib in its effects on proteasome and toxicity profile.[45] A major advantage of NPI-0052 over Bortezomib consists of its oral bio-availability. Studies performed in animal tumour models have indicated that NPI-0052 is well tolerated, prolongs survival and reduces the incidence of relapse, and acts synergistically with Bortezomib in causing an anti-MM effect.

3.3. *Nutlins*

Nutlins are recently developed small molecules acting on the p53/mouse double minute 2 (MDM2) E3 ubiquitin ligase module. In normal conditions, the oncosuppressor p53 is associated with and inhibited by MDM2, which blocks its transactivation domain and promotes its ubiquitin-dependent proteasomal degradation. p53 is one of the most frequently mutated tumour suppressor genes, as highlighted by its mutation in more than 80% of all human cancers. Therefore, p53 represents an ideal target of innovative anti-cancer therapies.[46,47]

Nutlins are non-genotoxic compounds which selectively bind MDM2 in the p53-pocket binding. As a result, nutlins effectively disrupt the p53/MDM2 interaction and stabilise p53, thus allowing for efficient activation of the p53 pathways. Recent pre-clinical studies have reported that Nutlin3 is able to kill CD19+ B-CLL cells but not normal CD19+ lymphocytes, peripheral blood mononuclear cells or bone marrow haemopoietic precursors.[48] The molecule induces p53 activation, which in turn determines activation of Cytochrome C-mediated apoptosis. In addition, Nutlin3 acts synergistically with both Chlorambucil and Fludarabine in determining B-CLL apoptosis.[48,49] A more recent study has pointed out that Nutlin3 induces p53-mediated apoptosis through transcription-dependent (enhancing transcription of Bcl-2 family proteins) and independent pathways (resulting from mitochondrial p53 translocation).[50] Nutlin and Fludarabine act synergistically enhancing p53 levels even in samples resistant to Fludarabine alone. Recent data suggest that Nutlin is also effective in determining programmed cell death of AML and MM cells. The apoptosis of AML blasts was enhanced when Nutlin was combined with cytosine arabinoside and Doxorubicin, suggesting a synergistic effect.[51]

3.4. *Heat shock protein (HSP) inhibitors*

A particular family of proteins which cooperate with the Ub-proteasome system in the quality control of cellular proteomes, ensuring proper folding, intracellular transport and repair or degradation of mis-translated, mis-folded or aged proteins, are heat shock proteins (HSP). These proteins are typically induced after cellular heat shock and are modulated by nutrient deprivation and oxidative stress. HSPs can bind to a multitude of proteins, called 'clients', forming multi-molecular complexes that either stabilise the client or assist its Ub-dependent degradation, for instance by recruiting the CHIP E3 ligase. [52]

Since HSPs act on caspase-dependent and independent apoptosis of tumour cells they are ideal targets of therapies aimed at modulating programmed cell death. Following this concept, several semi-synthetic inhibitors of HSPs have been developed that show clinical anti-tumour activity. Geldanamycin and 17-AAG are benzoquinonic ansamycins that target HSP90, interrupting its intrinsic ATPase activity, which drives the HSP90-based chaperone machinery. This results in degradation of the 'client' protein. [53] Geldanamycin has a high anticancer activity but presents an important hepatotoxicity so that it has been rarely employed in clinical investigations. 17-AAG, on the other hand, is less toxic even if it also presents several limitations (solubility, stability and hepatotoxicity). 17-AAG activity has been tested on leukaemia cell lines. It has been observed that the drug induced caspase-3 mediated apoptosis only in HSP90 highly positive cells; cells mildly positive were unaffected by the drug as well as normal haemopoietic progenitors.[54,55] 17-AAG activity is mild if the drug is employed as a single agent, whereas it is higher if it is combined with conventional chemotherapy schedules. This datum was confirmed by the observation that low doses of Geldanamycin were sufficient to induce apoptosis of Ph-positive acute lymphoblastic leukaemia (ALL) cells even if combined with ineffective doses of Doxorubicin.[56] Moreover,

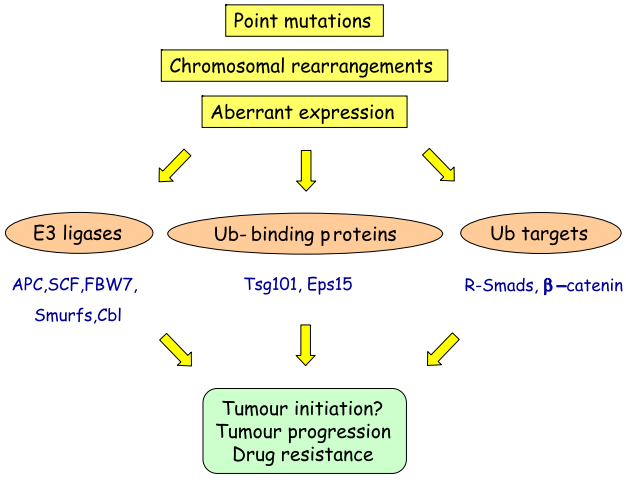

Fig. 3 – Cancer-associated mutations affecting the Ub system. Different types of somatic gene mutations, including point mutations and gross chromosomal rearrangements, have been found in multiple malignancies. Most of them affect either E3 ubiquitin ligases or Ub target proteins. With very few exceptions, solid proofs for a direct causative role of Ub system mutations in cancers are missing. More likely, loss of ubiquitin regulation, in the background of already active oncogenic signalling pathways, may contribute to some tumour phenotypes, including acquired resistance to chemo- and radio-therapies.

in vitro studies have shown that the addition of HSP90 inhibitors to conventional chemotherapy is able to overcome p53-mediated drug resistance.

Recently, it has been reported that Shepherdin, a new HSP90 inhibitor, uses different contact sites in the HSP90 ATPase pocket, is more specific and more potent than 17-AAG. Shepherdin has at least two anti-cancer mechanisms consisting of a quick disruption of mitochondrial function and a decreased expression of HSP90 client proteins Akt and CDK-6. In contrast to 17-AAG, shepherdin does not increase HSP70 levels in AML cells, therefore enabling administration for longer time periods.[57]

4. Conclusions

Alterations of several components of the ubiquitin-proteasome system have been described in multiple cancers (Fig. 3).[13] Alterations include gene loss or overexpression, point mutations as well chromosomal rearrangements. In most cases, such alterations involve either Ub modifying enzymes, such as E3 ligases, or ubiquitin targets themselves, making them resistant to ubiquitylation. We are not aware of any existing proof of any cancer-associated mutation inside a UBD, though the existence of such mutations in other diseases, as in the case of the UBA-containing proteins p62/Sequestosome and Paget's disease, suggests that this may well be the case. However, despite the growing number of alterations being described, there is little, if any, strong evidence supporting a direct, causative role for them in the process of oncogenesis. Instead, it is likely that aberrant Ub-mediated regulation of key oncoproteins or oncosuppressors may contribute to several characteristics of human cancers, including drug sensitivity. Therefore, targeting ubiquitin in malignancies has become a fertile land for drug development and the results of several clinical trials already support research in this direction.

Conflict of interest statement

None declared.

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