ITI Life Sciences

Market Foresighting
March 2007

Emerging Drug Targets:
Ubiquitin Signalling

Contents

Contents

Executive Summary

Executive Summary (1)

- Prior ITI-LS analysis of trends in Emerging Drug Targets in 2006 revealed Ubiquitin Ligases as a target of increasing interest. Given that ubiquitin ligases form part of a complex signalling pathway (the ubiquitin proteasome system, UPS) ITI-LS has expanded its focus to the major potential drug targets within the UPS
- The UPS was discovered in 1980 and implicated in protein degradation. A resultant Nobel prize in 2004 coincided with its implication in a range of additional key cellular functions and disease
- The UPS provides a rich source of emerging drug targets, yet it remains an unexploited and largely uncharted area for drug discovery
- The only approved UPS related therapeutic, Velcade (a proteasome inhibitor) has demonstrated proof of concept for UPS inhibition yet has multiple undesirable side effects associated with its broad ranging effects on general protein degradation

Executive Summary (2)

- Additional potential drug targets upstream of the proteasome such as E1 ubiquitin activating enzymes, E2 ubiquitin conjugating enzymes, E3 ubiquitin ligases and deubiquitinating enzymes (DUBs) present further opportunities for the development of therapeutics with increased specificity and selectivity and hence the potential for increased efficacy and reduced side effects
- Of these drug targets E3 ligases have been the most obvious targets for therapeutic intervention due to their primary role in controlling substrate specificity within the UPS. To date around 600 putative E3 ligases have been identified, creating a vast pool of potential therapeutic targets
- However, due to the complex multiple protein-protein interactions that would need to be modulated in the UPS, small molecule inhibition strategies remain problematic for most targets. Modulation via biologics including peptides, antibodies and RNAi may serve as further drug discovery options, although these offer additional challenges with respect to formulation, delivery and cost

Executive Summary (3)

- DUBs are emerging as a more favourable target class due to their protease function being more amenable to small molecule drug discovery and their previous exploitation by the Pharma industry
- Any decision on the appropriate drug target for exploitation will involve a trade-off between disease linkage, selectivity, sensitivity, and druggability of the target together with the level and quality of market competition
- From an initial scan of Scottish competencies in this area, it is clear that a number of parties possess some key building blocks which would be essential for impacting drug discovery on this emerging drug target area

Prior ITI Analysis of Emerging Drug Targets Area

Why Look at Emerging Drug Targets Now ?

A range of forces impacting on drug discovery and development are driving Pharmaceutical companies to search for new clinical treatments including:

- A dearth of novel chemical entities in Pharmaceutical pipelines
- Many me-too compounds and copycat products
- A lack of PII-PIII licensing candidates to fill late stage pipelines
- Increasing competition for late stage clinical assets is forcing the pharmaceutical industry to reach out to earlier stage opportunities to resolve their needs
- Consequently, the market appetite for novel leads/preclinical/early stage clinical compounds is high, leading to earlier stage licensing deals for leads against validated targets

In addition, the identification of future emerging drug targets presents multiple opportunities for commercial exploitation within various timeframes, including the development of tools/reagents, assays, animal models, diagnostics and therapeutics

ITI-LS therefore initiated Foresighting of the Emerging Drug Targets area, initially through the production of an Environmental Scan in 2006 that aimed to identify future trends in drug discovery and potential emerging drug targets

The Emerging Drug Targets Environmental Scan Identified Ubiquitin Ligases as a Growth Area

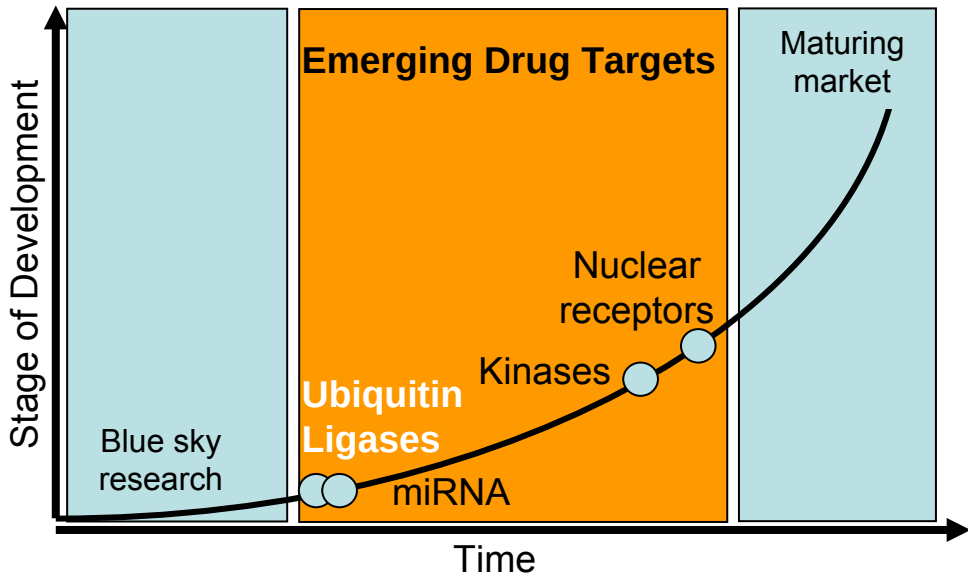
The previously published Emerging Drug Targets Environmental Scan identified 4 potential drug target classes of interest:

- Ubiquitin ligases
- miRNA
- Nuclear receptors
- Kinases

Based on ITI requirements for generating foreground intellectual assets, we decided to concentrate our initial focus on the *early* stage targets (ubiquitin ligases and miRNA)

From the E-scan, Ubiquitin ligases consistently stood out with regards to both trends in publications, patents analysis and discussions with key opinion leaders and scientific advisory board members

We have therefore expanded ubiquitin ligases to include additional potential targets within the ubiquitin signalling system for the scope of this Foresighting report



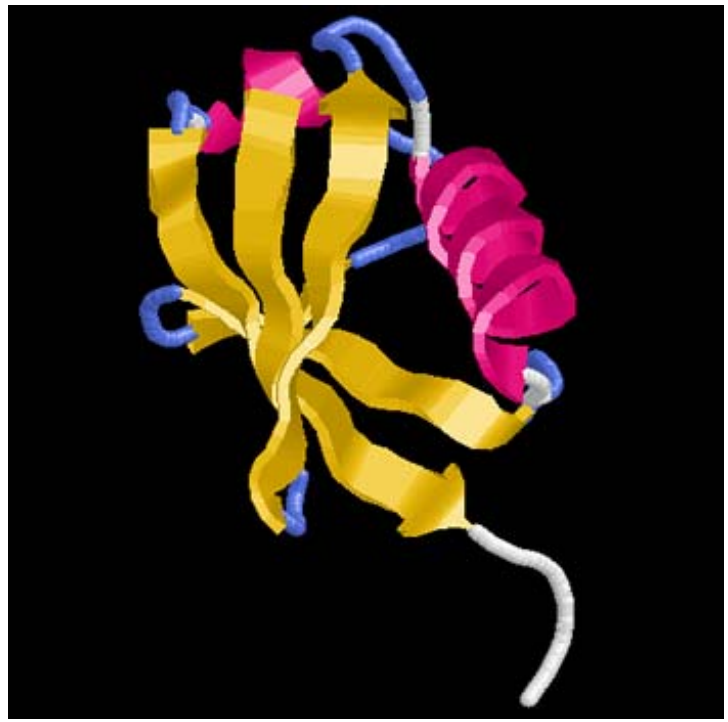
The orange zone represents the ITI-LS sweet spot for Emerging Drug Targets

Introduction to Ubiquitin

Introduction to Ubiquitin

Ubiquitin is a highly conserved 76 amino acid / 8.5kDa polypeptide first discovered in 1975 and found to be ubiquitously expressed in all eukaryotic cells - see sequence below

Ubiquitination is a post translational modification that involves the covalent attachment of one or more ubiquitin molecules via the formation of an isopeptide bond between the C-terminal glycine on Ubiquitin and a corresponding lysine residue on a target protein



Ubiquitin has a very compact structure consisting of two α-helices and two β-sheet structures. The C-terminus of ubiquitin is extended and unstructured

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

Amino acid sequence of ubiquitin

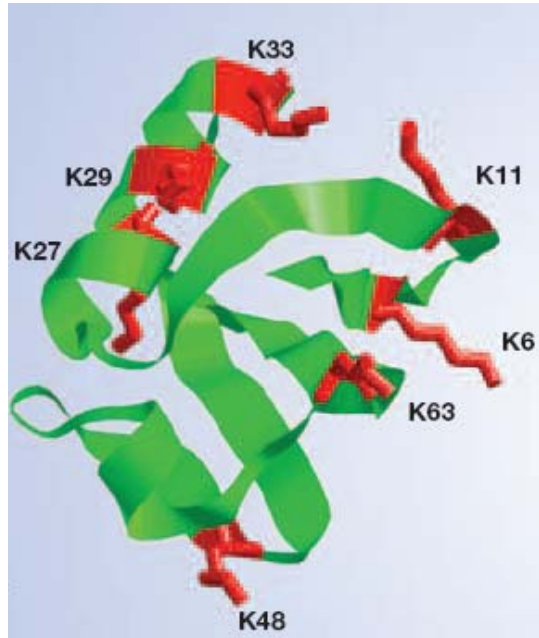
Consequences and Control of Ubiquitination

Initial studies have implicated ubiquitination as one of the primary routes for protein degradation. However, recent studies have demonstrated broader roles for ubiquitin in cellular control mechanisms

Importantly, the physiological effects of ubiquitination are dependent upon:

- The number of ubiquitin molecules. Substrates can have single or multiple ubiquitin molecules attached via lysine residues, referred to as mono-, multi- or poly-ubiquitination respectively
- The location of the lysine residue on ubiquitin involved in poly-ubiquitin chain extension. Ubiquitin contains 7 lysine residues which are implicated in poly-ubiquitination, as highlighted in the amino acid sequence of ubiquitin shown below and the diagram on the right

It is now becoming clear that the level of ubiquitination influences and controls key cellular processes, suggesting that modulation of the ubiquitin proteasome system (UPS) presents a novel strategy for drug discovery across many disease states



Ubiquitin has 7 lysine residues each of which can function as potential conjugation sites

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

Mono- and Multi-ubiquitination

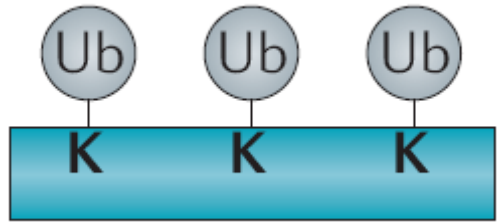
Mono-ubiquitination is the addition of a single ubiquitin molecule onto a substrate protein to:

- Regulate substrate activity instead of enforcing degradation
- Modify protein function by acting as a bait, allowing proteins that contain ubiquitin binding domains (UBDs) to attach and interact with the substrate
- Influence sub-cellular localisation and cellular signalling pathways such as those involved in endocytosis, endosomal sorting, histone regulation, virus budding, nuclear export and DNA repair

Multi-ubiquitination is the addition of ubiquitin molecules to multiple lysine residues on the same protein substrate

- Associated roles include substrate degradation and control of endocytotic mechanisms

A single ubiquitin molecule bound to a protein substrate via lysine linkage

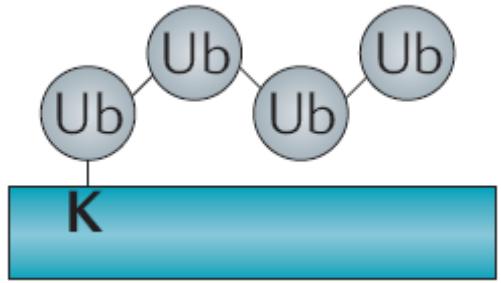


Single ubiquitin molecules bound to a protein substrate via multiple lysine linkages

Poly-ubiquitination

Poly-ubiquitination is the addition of multiple ubiquitin molecules to a single lysine residue on a substrate protein.

- It is a major control mechanism for regulatory proteins that require a rapid on/off mechanism. These include signalling pathway components, cell cycle regulatory proteins and proteins involved in stress responses



Multiple ubiquitin molecules bound to a protein substrate via a single lysine linkage

The ubiquitin molecule serves as the point of further chain extension via formation of additional isopeptide linkages with the C-terminus of ubiquitin through lysine residues located on the ubiquitin molecule itself

- Ubiquitin contains seven lysine residues. The most prominent site of ubiquitin chain extension is lysine-48 (K48) which is the primary form that is recognized by the proteasome leading to degradation
- However, other chain linkages, including K63-linked polymers, have also been identified which have a broader regulatory function rather than facilitating degradation

Ubiquitin-like proteins (UBLs)

Eukaryotic cells have evolved additional ubiquitin-like signalling systems, utilising ubiquitin-like proteins (UBLs) that share the same fold as ubiquitin

The UBLs are conjugated to their target proteins in a manner that mechanistically resembles ubiquitination but have dramatically different functional consequences

Examples of such ubiquitin-like proteins (UBLs) include:

SUMO

- Sumoylation is implicated in nuclear cytoplasmic transport, DNA repair, chromatin remodelling and regulation of transcription. Known targets include p53, IκB and caspase8

NEDD8

- Neddylation is linked with control of specific ubiquitin E3 ligases

ISG15

- ISGylation is associated with regulation of the immune response

From an emerging drug target perspective, the broad array of control conferred by variations in ubiquitin and ubiquitin-like/substrate binding adds complexity and opportunity for exploitation

Introduction to the Ubiquitin-Proteasome System (UPS)

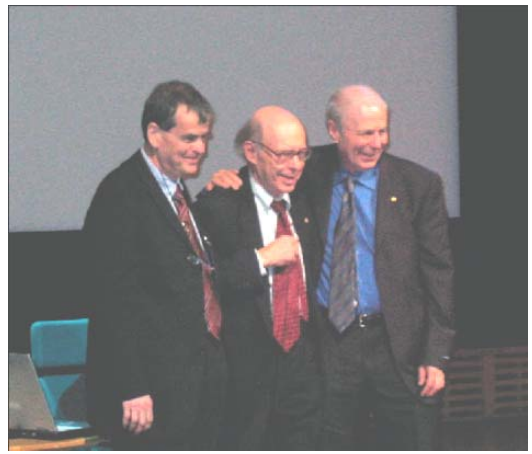
Discovery of the Ubiquitination System

The ubiquitination system was initially characterised as an ATP-dependent proteolytic system present in cellular extracts in 1980 by Irwin Rose, Avram Hershko, and Aaron Ciechanover, who were awarded the 2004 Nobel Prize in Chemistry for the discovery of ubiquitin-mediated protein degradation

A heat stable polypeptide, termed ATP-dependent proteolysis factor 1 (APF-1), later identified as ubiquitin, was found to become covalently attached to substrates in an ATP and Mg^{2+} dependent process

Following binding of multiple APF-1 molecules to a single protein substrate via isopeptide linkages, the substrate was degraded with the concomitant release of free APF-1

Subsequent research has led to the detailed understanding of the three key steps involved in the ubiquitination process, which are described as follows



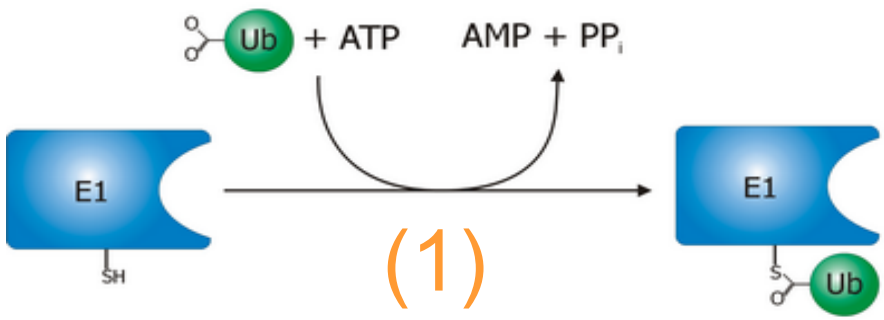
The laureates at the Karolinska Institute after their Nobel addresses. Shown are (left to right) Aaron Ciechanover, Irwin Rose, and Avram Hershko.

The UPS – E1 Ubiquitin Activating Enzyme

The process of marking a protein with ubiquitin consists of a series of 3 key steps:

1. ***Activation***

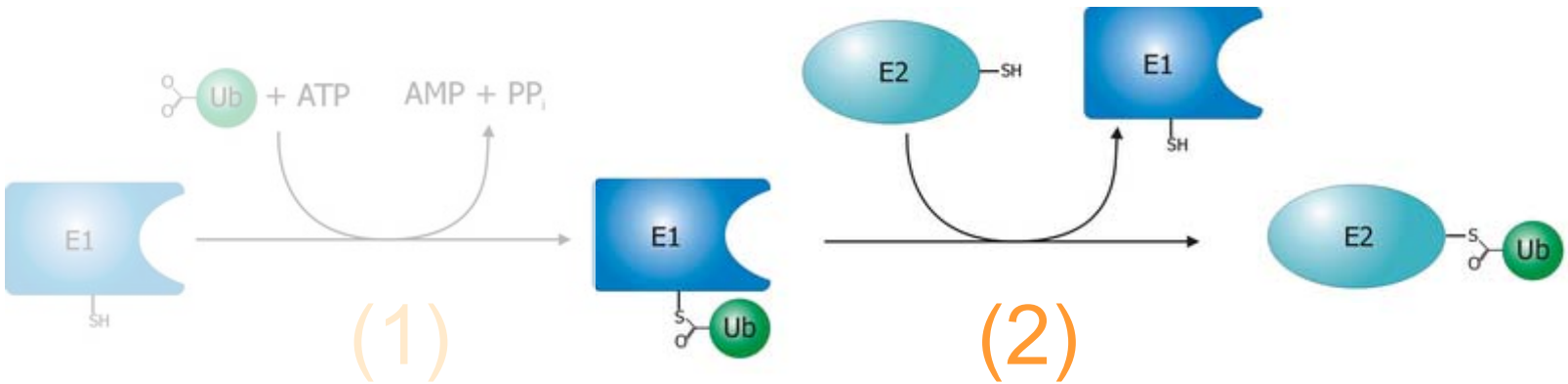
   Ubiquitin is activated for subsequent conjugation via a 2-step process in an ATP-dependent reaction involving a single E1 ubiquitin activating enzyme. The first step in the UPS involves the production of a ubiquitin-adenylate intermediate, whilst the second step transfers ubiquitin to the E1 active site cysteine residue, with release of AMP. This step results in a thioester linkage between the C-terminal carboxyl group of ubiquitin and the E1 cysteine sulphydryl group



The UPS – E2 Ubiquitin Conjugating Enzyme

2. Conjugation.

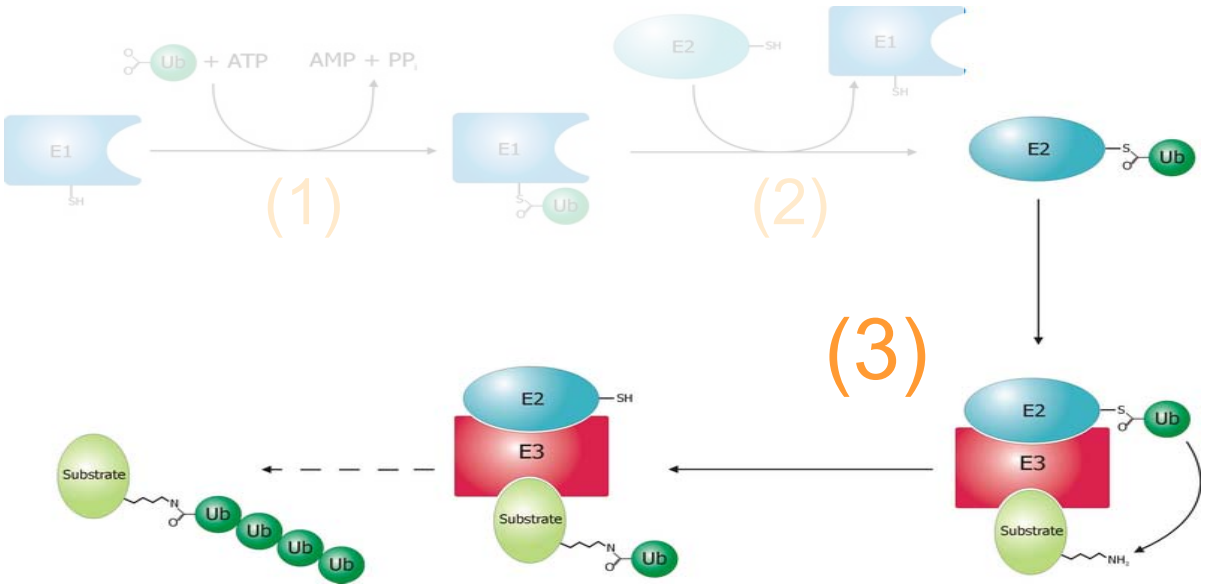
The second step in the UPS involves the transfer of the ubiquitin from the E1 ubiquitin activating enzyme to the active cysteine of an E2 ubiquitin conjugating enzyme via a trans(thio)esterification reaction.



The UPS – E3 Ubiquitin Ligase

3. Ligation.

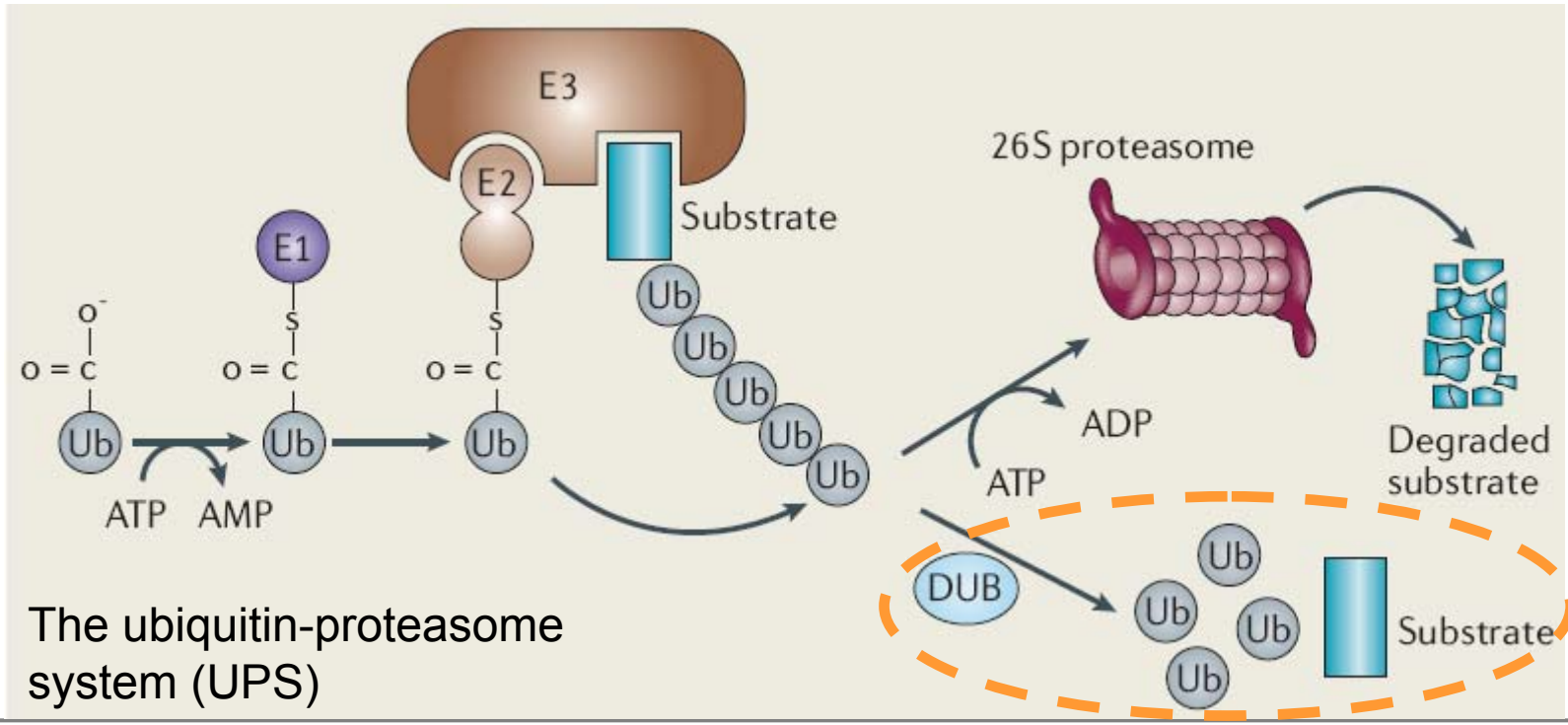
The final step involves the binding of the E2 to a specific E3 ligase, which has previously bound the target substrate, therefore bringing it into the vicinity of the E2-ubiquitin complex. Transfer of the ubiquitin to the target substrate can occur either directly from the E2 (as shown below), or via an E3-ubiquitin intermediate. The end result is an isopeptide bond between the substrate lysine and the C-terminal glycine of ubiquitin. Multiple ubiquitin molecules may subsequently be added



The UPS – Deubiquitinating Enzymes (DUBs)

Following poly-ubiquitination of the protein substrate, the most likely result is the targeting of the substrate to the proteasome for degradation with the concomitant release and recycling of the ubiquitin molecules via deubiquitinating enzymes (DUBs).

These enzymes can also rescue ubiquitinated substrates from degradation by removing the ubiquitin molecules prior to the substrate reaching the proteasome which highlights the reversible nature of the UPS



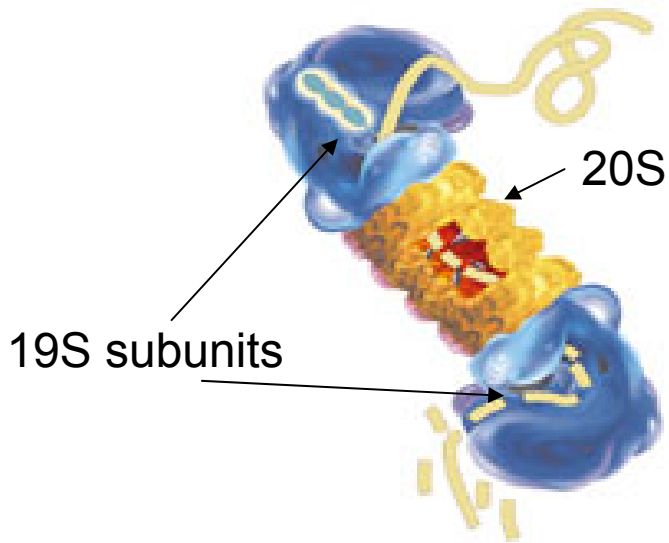
The ubiquitin-proteasome system (UPS)

The UPS – The Proteasome

Poly-ubiquitination leads to targeting of the substrate to the proteasome, a large complex with a 20S barrel-shaped proteolytic core and two 19S regulatory "caps" at either end

- The 19S caps recognize the poly-ubiquitin chain as a signal to de-ubiquitinate (ubiquitin molecules are then recycled) and unfold the target substrate in an ATP-dependent manner
- The unfolded substrate is then pulled through the hollow core of the 20S catalytic centre where the protease active sites are located
- Following degradation of the substrate, the peptides generated are used in antigen presentation or degraded to amino acids that are recycled for new protein synthesis

IN: intact ubiquitinated protein



OUT: small peptide fragments

The proteasome also degrades misfolded, damaged or mutant proteins that could be harmful to the cell

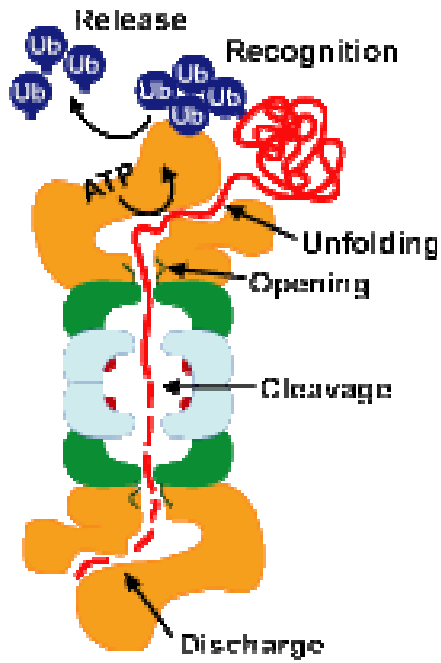
Positive and Negative Regulation of Protein Degradation

The disease states associated with protein degradation in the UPS can be classified into two groups:

1.Those that result from loss of function leading to stabilization of associated proteins, i.e. proteins do not get degraded

2.Those that result from gain of function leading to abnormal or accelerated degradation of the protein substrate

A better understanding of the processes involved in degradation of key disease-associated regulatory proteins, or indeed proteins linked with other cellular controls aside from degradation, may lead to the development of mechanism-based drugs with target specificity



Proteasomal degradation of a ubiquitinated substrate

Drug Discovery in the UPS

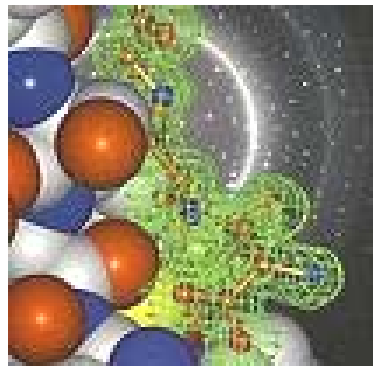
Drug Discovery in the UPS

So far we've described ubiquitin, the UPS and its individual components….

….now we want to expand upon the specific dynamics influencing UPS drug discovery, potential sites of modulation and highlight the scope for emerging drug targets within the UPS, at the present time

What Makes a Good Drug Target?

When considering potential drug targeting strategies, there are a range of critical factors which must be considered, including:



Druggability

- How easily a target can be modulated by small molecules and/or biological approaches

Sensitivity

- The required affinity and efficacy of any administered agent to modulate the target

Selectivity

- The potential to develop agents that can preferentially modulate one target versus another

Pharmacokinetics & ADMET

- Can any administered agent be developed that has appropriate Absorption, Distribution, Metabolism, Excretion and Toxicity profiles to modulate the target without causing unacceptable adverse events

Points to bear in mind when targeting the UPS

Disease linkage is critical

- The role of the UPS in disease is really in its infancy, as only a small fraction (<20%) of the genes with potential links to the pathway based on known sequence motifs have been studied
- Is the drug target (over, or under) expressed in the appropriate diseased tissue?

Drug discovery in the UPS may be initiated by either:

- Developing inhibitors against the potential targets and then using these to link to disease states, or
- Elucidating the link between the target and disease prior to developing inhibitors

What is the nature of the pathway alteration that one is trying to modify?

- For example, it might be easier to inhibit a hyper-active or over-expressed component of the pathway than to resurrect activity from a mutant protein with reduced or negligible activity

How particular classes of proteins within the UPS can be targeted

- Some components of the UPS might be more amenable to small molecule modulation than other components of the system

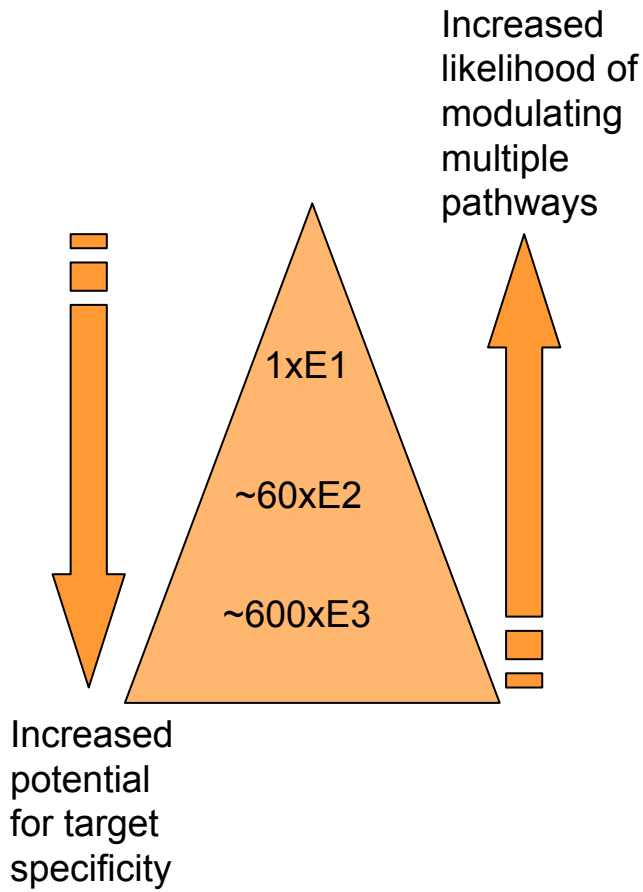
How specific for a given target does a therapeutic agent need to be?

- Because many components in the pathway are from multi-gene families, attention to specificity is required, as off-target effects can reduce the suitability of particular classes of inhibitors

The UPS is Hierarchical

The organisation of the UPS is hierarchical

- One common E1 activating enzyme activates ubiquitin for all cellular ubiquitination networks
- Multiple combinations of around 60 E2 conjugating enzymes and at least 600 E3 ligases are used to catalyze ubiquitination of many more substrates in a target-specific manner
- It is this carefully regulated pattern of interactions between E3s and their targets that provides the specificity necessary for appropriate control of ubiquitinated substrates
- Similar hierarchical systems are also involved in ubiquitin-like (UBL) pathways with varying degrees of complexity



From a therapeutic point of view, this provides the opportunity to modulate the UPS at multiple sites with varying degrees of target selectivity dependent upon which of the components of the UPS is targeted

Proteasome Inhibition as the First Target Area in the UPS

The UPS plays an essential role in up-regulation of cell proliferation (for example through polyubiquitination of cyclins), down-regulation of apoptosis (through degradation of caspases), and development of drug resistance in many human tumour cells, suggesting the use of proteasome inhibitors as potential novel anticancer drugs

The most clinically advanced inhibitors of the UPS directly target and inhibit the 20S proteasome, the core of the proteolysis machinery, rather than the upstream components of the UPS

Proteasome inhibitors are broadly categorized into two groups:

- Synthetic analogs. Synthetic proteasome inhibitors target serine and cysteine proteases, therefore inhibiting the proteolytic activity within the proteasome
- Natural products. Activity has been found in yeast and bacteria metabolites that irreversibly modify the 20S proteasome

However, as the proteasome is involved in a variety of biologically important functions, it is of particular interest to block the proteasome for a limited time in order to reduce cytotoxic effects

Velcade Validates the UPS as a Pathway for Drug Discovery

Although there are potentially many different components of the UPS that might be targeted for inhibition of a particular disease, the first success in the clinic has come from the inhibition of the proteasome itself

Bortezomib (Velcade; Millennium and J&J), a proteasome inhibitor, was approved in May 2003 for the treatment of Multiple myeloma

The relative selectivity of proteasome inhibition for killing tumour cells as opposed to normal cells was unexpected

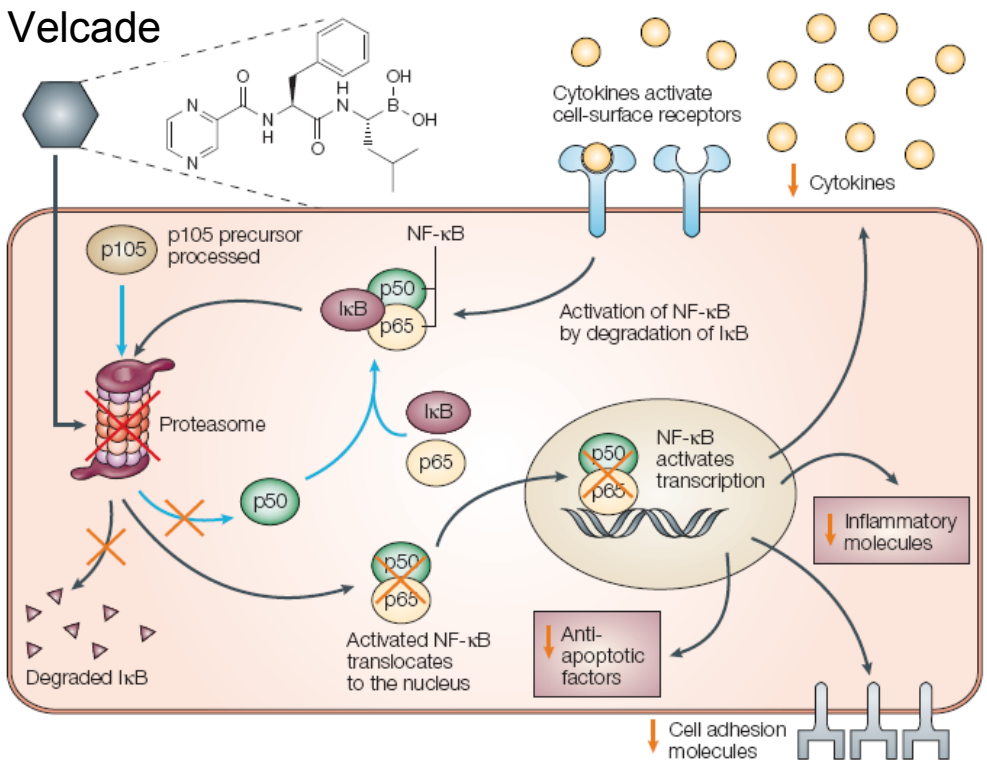
- However, this selectivity can now be rationalized based on the principle that tumour cells generate higher concentrations of aberrant proteins as well as higher amounts of onco-proteins, making them more sensitive to the effects of proteasome inhibition

Nevertheless, the success with proteasome inhibition, at least for certain types of cancer, suggests that the development of more specific compounds targeting the UPS could be clinically important

Mode of Action of Velcade

A key factor in the ability of Velcade to kill myeloma cells is in blocking activation of NF-κB

- In normal cells, NF-κB is bound to the inhibitory protein IκB, which maintains it in the inactive form in the cytosol
- Certain tumours have activated forms of NF-κB, and the proteasome is essential for this activation
- By inhibiting the proteasome and therefore the activation of NF-κB, Velcade helps to reduce: anti-apoptotic factors; inflammatory molecules; cell adhesion molecules, which allow attachment cells to adhere to bone marrow cells; and cytokines, which promote the growth of myeloma cells



But Velcade is Far From Perfect

Due to proteasomal inhibition targeting the final step in the UPS, Velcade is thought to lack target specificity which has implicated it with a broad spectrum of undesirable side effects including:

- Nausea, vomiting, fatigue, diarrhoea, dehydration, constipation, fever, loss of appetite, headaches, dizziness, peripheral hypotension, lowered resistance to infection, low platelet count, peripheral neuropathies, muscle cramps/painful limbs, insomnia, confusion, anxiety, blurred vision, oedema, heart problems and liver or kidney problems

As such, Velcade is only indicated for the treatment of patients who have received prior therapies and have demonstrated disease progression on recent therapy

Because of patient restrictions, the total sales estimates for Velcade will not change dramatically from a baseline of approximately $200 million unless approval for additional indications is forthcoming

Although unproven, targets upstream of the proteasome such as E1, E2 and E3 may offer greater specificity with reduced side effect profiles

Drug Discovery in the UPS

E1 Ubiquitin Activating Enzymes as Drug Targets

E1 Ubiquitin Activating Enzyme

The E1 ubiquitin activating enzyme represents the first step in the UPS and therefore may be attractive as a potential drug target

However, given its hierarchical position in the UPS, the E1 enzyme initially appears to have limited therapeutic potential due to its broad effects on multiple E2 conjugating enzymes

Co-analysis of ubiquitin-like E1 enzymes as additional drug targets is currently ongoing in an attempt to understand the structural biology underpinning potential inhibition strategies in the following:

- SUMO E1
- NEDD8 E1
- ISG15 E1

Whilst the crystal structure of Ubiquitin E1 has not yet been resolved, both the SUMO E1 and the NEDD8 E1 crystal structures have been elucidated and are driving greater understanding of the critical regions involved in ubiquitin activation and drug targeting

Structural Analysis Leads to Identification of E1 Inhibition Strategies

Both SUMO and NEDD8 E1 enzymes are heterodimeric complexes that contain domains which contribute substantially to the ATP-binding site responsible for adenylation of ubiquitin

By contrast, Ubiquitin E1 represents a fusion of these two proteins to form a single polypeptide which is expected to form a similar 3D structure

In addition to the adenylate pocket described above, two additional functional domains are found in the complexes:

- A catalytic cysteine residue, which is the site of thiol ester formation with the UBL.
- A ubiquitin-like fold domain that is important for interaction with E2

Possible Mechanisms for Inhibiting E1 Enzymes

This structural analysis to date has resulted in two possible ways of inhibiting E1 enzymes:

1. The identification of inhibitors of ubiquitin or ubiquitin-like adenylation by either blocking access of ubiquitin or the UBL to the adenylation site, or by blocking access of ATP
   - This is a potentially attractive route for drug discovery due to the previous knowledge gained in the identification of kinase inhibitors that target the ATP binding site

2. The identification of inhibitors that block the interaction of E1 with E2
   - This may be less attractive to small molecule drug discovery due to the large protein-protein interaction surfaces involved

E1 Ubiquitin Activating Enzymes as Drug Targets

In developing E1 enzymes as drug targets, it is critical to understand potential physiological consequences of inhibition to guide therapeutic application

- Previous studies in mammalian cells indicate that mutations in Ubiquitin E1 arrest the cell cycle in late S phase and G2, indicating that it is required for cell proliferation
- Similarly, mutations in NEDD8 E1 effectively block cell division in *C. elegans*
- One potential advantage of inhibiting NEDD8 E1 over ubiquitin E1 is that the only validated function for NEDD8 is activation of the Cullin class of E3 ubiquitin ligases (see page 50), several of which are candidate targets for drug discovery relatively reducing potential side effect liabilities

In principle, E1 inhibitors might provoke cell-cycle arrest and could be useful in hyperproliferative disorders such as cancer

- However, it is likely that multiple pathways would be affected and many of these pathways will be important for the functions of normal cells
- Still, it remains to be determined whether a sufficient therapeutic window can be attained with an inhibitor of the E1 class of enzymes.

As with proteasome inhibition, through Velcade, E1 ubiquitin activating enzymes as a drug target area may suffer from a lack of specificity through its hierarchical position in the UPS

Drug Discovery in the UPS

E2 Ubiquitin Conjugating Enzymes as Drug Targets

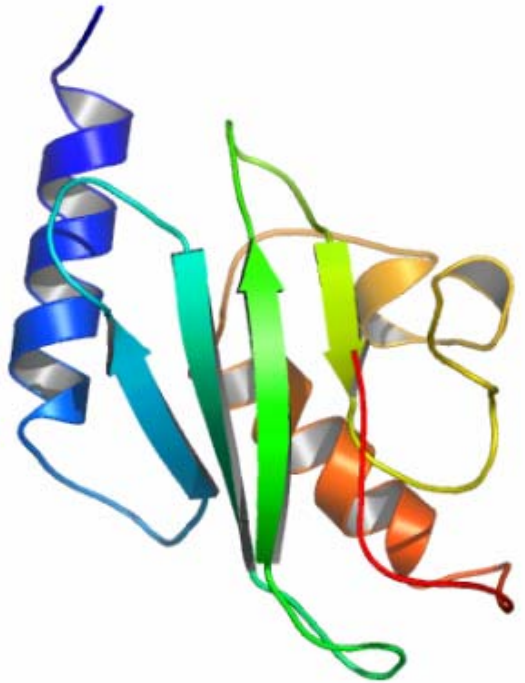
E2 Ubiquitin Conjugating Enzymes

E2 ubiquitin conjugating enzymes represent a second targetable step in the UPS

Unlike E1 ubiquitin activating enzymes, E2 conjugating enzymes potentially represent a target area of greater selectivity with around 60 family members described in mammals to date

Of these, four classes have been described, each sharing a core catalytic domain (termed UBC) required for ubiquitin conjugation

The E2 enzyme class is structurally the most studied group in the ubiquitination pathway and is composed of a highly conserved fold consisting of a core of beta strands flanked by N- and C-terminal alpha helices. A further defining E2 feature is the catalytic cysteine (Cys85) which forms a thioester bond with ubiquitin located in a groove on the loop between the beta strand mesh and the ensuing helix.



The crystal structure of human Ubiquitin Conjugating Enzyme E2 UEV1

Structural Analysis Leads to Classification of E2 Enzymes

Each E2 can be classified on the basis of their core catalytic domain (UBC) into four classes:

Class I enzymes consist of just the UBC. *In vitro* these enzymes are very poor at transferring ubiquitin to proteins on their own, and probably require an E3 to aid this *in vivo*. UBC 4 and 5 of *S. cerevisiae* and UBC1 of *A. thaliana* are examples of this class of E2, and are known to be important in the ubiquitination of many short-lived and abnormal proteins prior to degradation

Class II enzymes possess a UBC and a C-terminal extension. The extensions are different in type and appear to mediate interaction with protein substrates and DNA repair. These interactions may be a form of ubiquitination that results in protein modification but not degradation. Other C-terminal extensions appear to be involved in E2 localisation

Class III enzymes possess a UBC and an N-terminal extension. Several enzymes of this class have been identified but the function of the extensions is unknown

Class IV enzymes possess a UBC and both N- and C-terminal extensions. These extensions appear to be important for some subfamily function, including E2 localisation and protein-protein interactions

E2 Ubiquitin Conjugating Enzymes as Drug Targets

In developing E2 enzymes as drug targets, it is critical to understand potential physiological consequences of inhibition to guide therapeutic application

Reports indicate that Ubc9, the single SUMO E2 enzyme catalyzing the conjugation of SUMO to target proteins, is over-expressed in certain tumours, such as lung adenocarcinoma, ovarian carcinoma and melanoma, suggestive of therapeutic relevance

Ubc12, the single NEDD8 E2 enzyme has been implicated as an anticancer drug target through its role in controlling cell replication

- The discovery of a tail structure on Ubc12 has given light to new potential targeting mechanisms that distinguish it from concomitant ubiquitin E2 enzymes
- Knowing the exact shape and function of the E2 tail makes these structures potential targets for new drugs that might block the ability of the NEDD8 pathway to accelerate replication of cancer cells
- The conservation of E2 enzymes across the ubiquitin and ubiquitin-like protein pathways indicates that analogous tail structures of E2 enzymes may have similar roles

E2 ubiquitin conjugating enzymes have the potential to provide targets that have enhanced selectivity over E1 ubiquitin activating enzyme and proteasome targets but suitable targets and strategies have yet to appear

Drug Discovery in the UPS

E3 Ubiquitin Ligases as Drug Targets

E3 Ubiquitin Ligases

The E3s regulate much of the specificity within the UPS due to:

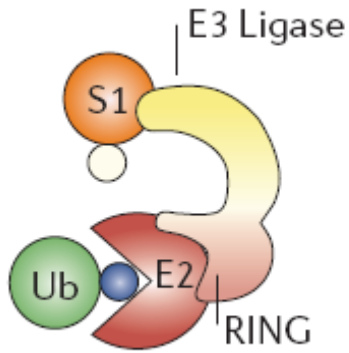
- Specific recognition and binding of protein substrates
- Bringing E2s into the proximity of these specific substrates

There are three major classes of E3, each with distinct protein interaction domains that bind specific E2s:

- RING-finger E3s (the largest class). These include the simple RING finger and Cullin-based RING finger E3s
- U-box E3s
- HECT-domain E3s
- Additional domains function to recruit specific protein substrates

The differentiators between the above classes are due to their distinct roles in ubiquitin transfer:

- HECT domains function directly in ubiquitin transfer by forming a thiol ester intermediate with ubiquitin, which is then transferred to substrate
- RING-finger E3s, or the closely related U-box E3s, do not function directly via an intermediate, but simply function as adaptors



RING-finger ubiquitin ligase

Advantages of targeting E3 Ligases

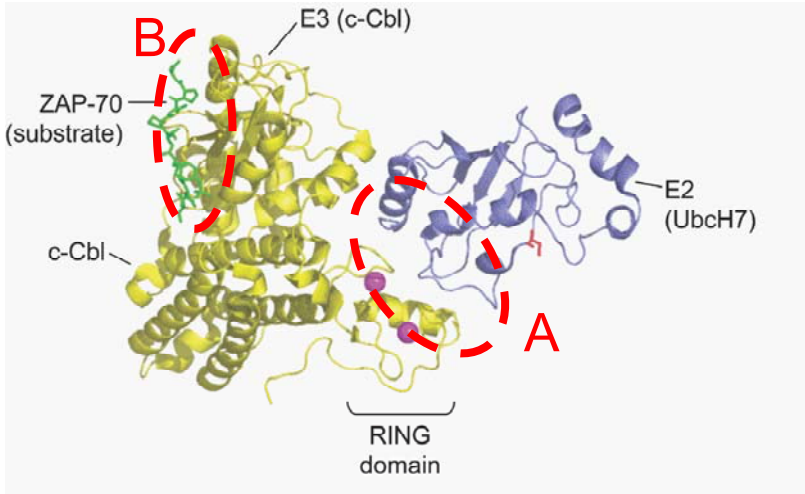
Since the E3 specificity modules determine which substrate is to be ubiquitinated, it might be feasible to regulate the activity of selected protein substrates by manipulating their specific E3s

The sheer number of E3s (~600) adds credence to this potential advantage

Additionally, multiple potential target sites exist at the binding regions (shown right) of both E3 to E2 (A) and E3 to protein substrates (B) providing further opportunities for drug targeting

While Velcade has a broad effect through proteasome inhibition, targeting a single E3 should allow for manipulation of distinct protein substrates

This increase in the specificity of therapeutic intervention could potentially boost the effectiveness of the treatment and eliminate some non-specific side effects at the same time



The crystal structure of an E3 ligase bound to a protein substrate and an E2 ubiquitin conjugating enzyme demonstrating potential binding regions that could be targeted (A and B)

E3 Ligases and Disease

As in other areas of UPS drug discovery, the link between E3s and specific disease states is at an early stage

Given the broad and diverse range of potential E3 ligase targets, this report will not review all mechanisms and targets exhaustively

For simplicity, focus will be on the largest class of E3 ligases containing:

Simple RING finger E3 ligases implicated in:

- Oncology
- Parkinson's Disease

Cullin-based RING finger E3 ligases implicated in:

- Oncology
- Inflammation

Simple RING finger E3 Ligases in Oncology

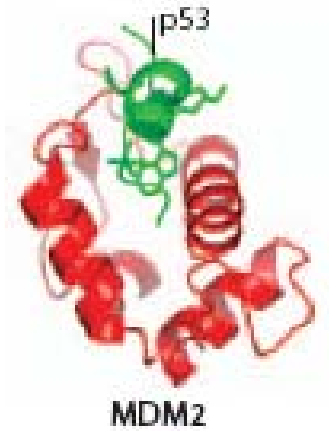
p53 & MDM2

p53 is a major tumour suppressor that is mutated in 50% of all human tumours

MDM2 is the major E3 ligase of p53, leading to p53 degradation

- MDM2 is an oncogenic simple RING finger E3 ligase that is over-expressed in various tumours
- MDM2 expression is transcriptionally induced by p53, enabling a negative feedback loop, controlling p53 degradation rates
- Structural analysis has demonstrated that MDM2 contains an open p53 binding pocket that may be accessible to small molecules

Inhibition of MDM2 should elevate wild type p53 levels driving damaged cancer cells into apoptosis



Simple RING finger E3 Ligases in Oncology

Useful strategies for stimulating the tumour suppressor activities of p53 include:

- Inhibition of the interaction between MDM2 and p53
- Inhibition of the ubiquitination of p53

From the above two strategies a number of chemical series have recently been discovered that demonstrate proof of concept:

Nutlins (cis-imidazoline derivatives)

- The first compounds demonstrated to block the interaction between p53 and MDM2
- They activate p53 dependent cell cycle arrest and apoptosis in cancer cell lines
- Nutlins are effective *in vivo* upon oral administration in nude mice tumour xenograft models

RITA (2,5-bis(5-hydroxymethyl-2-thienyl)furan)

- Found to have *in vivo* anti-tumour activity via a separate mechanism that involves preventing the recognition of p53 by MDM2

Simple RING finger E3 Ligases in Parkinson's Disease

Parkin is a gene that encodes a simple RING-finger E3 ligase

The gene is mutated in 50% of juvenile Parkinson's disease patients

- Its normal role is likely to be involved in protecting nerve cells from excitotoxic insults by acting as an E3 for currently unknown substrate proteins

Familial mutations suggesting that the UPS is required for Parkin's normal functionality have been discovered in:

- The RING finger (required for E2 binding)
- The ubiquitin-like domains (required for proteasome targeting) of Parkin

As such any therapy would need to be capable of promoting activity of a non-, or partially-functioning allele

Cullin-based RING finger E3s

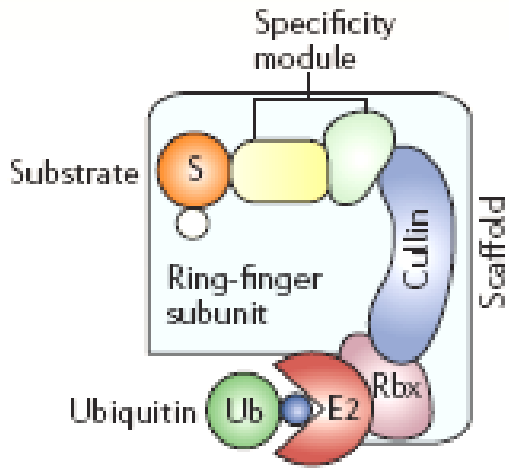
Cullin-based RING finger E3 complexes are multi-subunit ubiquitin ligases that consist of:

- A scaffold-like Cullin molecule (CUL1-5) that interacts with a RING-finger-containing subunit (RBX1 or RBX2), which binds specific E2s
- Interchangeable substrate-specificity modules, including F-box proteins which bind to CUL1, BTB proteins which bind to CUL3 and SOCS-box proteins which bind to CUL5

The human genome contains at least 68 F-box proteins, 170 BTB proteins and >45 SOCS proteins and each of them is likely to target multiple substrates for degradation

The complicated molecular networks linking Cullin-based RING finger E3s to their substrates is just starting to be deciphered

- Even for the most thoroughly studied CUL1-based SCF complexes, only a small number of F-box protein–substrate pairs have been identified
- Clearly, more research is needed to understand which proteins are targeted by specific SCF E3s and, consequently, to determine which SCF complexes deserve more attention from a therapeutic standpoint.



An example of a typical Cullin-based RING finger E3 ligase

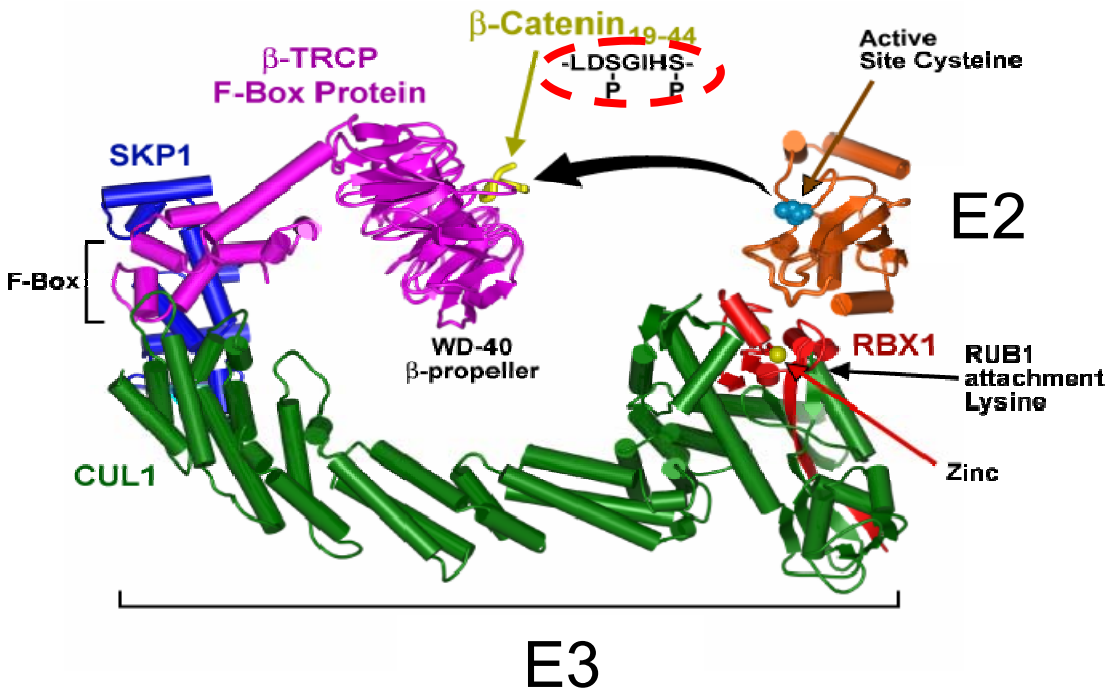
F-Box Proteins and Phosphorylation

Most F-box proteins (shown right in purple) interact with their targets (e.g. β-Catenin, shown in yellow) via specific domains through post-translational modification of the target, primarily via phosphorylation

These interaction domains include WD40 domains and leucine-rich repeats (LRRs) which represent phosphopeptide-interaction motifs

WD40 motifs have been shown to interact directly with phospho-degrons (small phospho-peptides, circled in red) which enhance specificity

Due to this specificity, understanding how substrates and F-box proteins interact has been an active area of research and is a potential area for drug discovery



Cullin-based RING finger E3 Ligase in Oncology

SCF^{SKP2}

SKP2 is an F-box protein that degrades several negative cell-cycle regulators, including the cyclin-dependent kinase (CDK) inhibitor p27. p27 is phosphorylated by CDKs to generate a phosphodegron that is recognized by SCF^{SKP2}

- The small CDK-interacting protein CKS1 is a subunit of the SCF^{SKP2} complex and is required for the interaction between SKP2 and p27 and subsequent p27 degradation
- SKP2 has also been linked with degradation of other cell-cycle regulatory molecules such as p130, p21 and p57 and FoxO, suggesting that SKP2 may be a putative proto-oncogene

The oncogenic potential of SKP2 has been confirmed via expression studies in mouse and human tumours, with overexpression of SKP2 correlating with loss of p27 expression

SKP2 therefore appears to be a valid therapeutic target with many drug discovery programmes active in this area. Down regulation of SKP2 activity might prove useful in the therapy of cancers associated with SKP2 overexpression

Cullin-based RING finger E3 Ligases in Inflammation

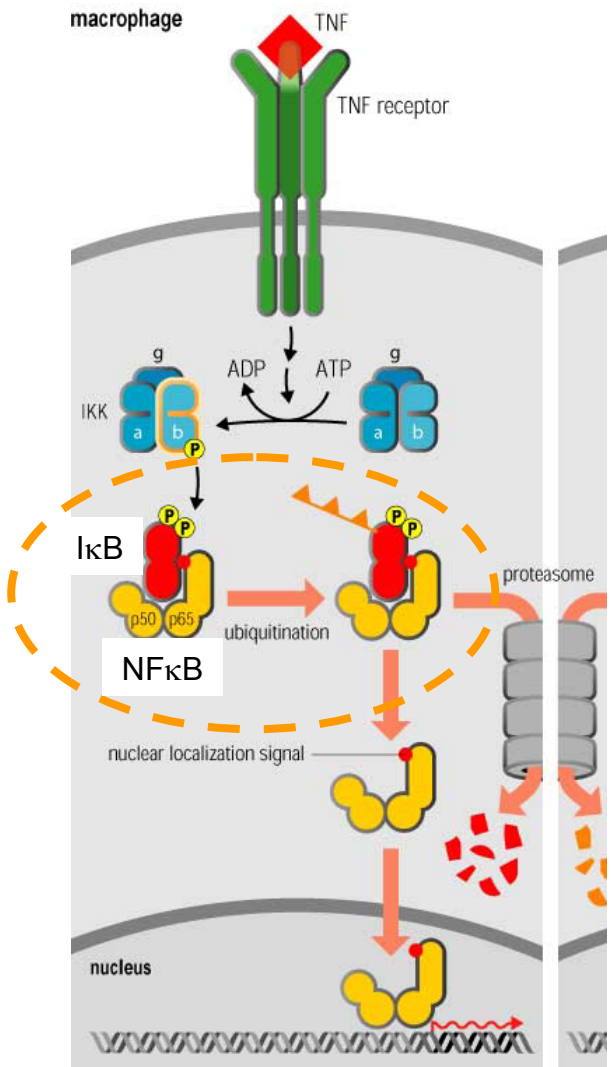
$SCF^{\beta TRCP}$

NF-κB is a transcription factor involved in the control of a large number of cellular processes, such as immune and inflammatory responses, developmental processes, cellular growth, and apoptosis. In addition, NF-κB is persistently active in a number of disease states, including cancer, arthritis, chronic inflammation, asthma, neurodegenerative diseases, and heart disease

$SCF^{\beta TRCP}$ is the E3 ligase for IκB which is the inhibitor protein for NF-κB

- Degradation of IκB releases NFκB from its inactive state, allowing translocation to the nucleus and transcription of genes involved in cytokine and survival responses
- Interaction of $SCF^{\beta TRCP}$ with IκB is through a phosphodegron sequence generated on IκB by an upstream kinase
- As a result $SCF^{\beta TRCP}$ is a potential target for anti-inflammatory agents

However, drug discovery efforts are hindered by the fact that β-TRCP promotes the ubiquitylation of multiple other substrates with similar phosphodegron sequences suggesting that development of inhibitors that are specific for one substrate may be challenging



E3 Ubiquitin Ligases as Drug Targets

Whilst, in theory, inhibitors of E3 ligases should be highly specific drugs with few side effects they have proven to be difficult drug targets thus far

This is primarily due to current scientific limitations when targeting:

- Protein-protein interactions, which are typically difficult to target with small molecules
- Multiple protein-protein interactions required for E3-substrate linkage

Additionally, multiple protein substrates are targeted by individual E3 ligases, which may lead to some undesirable/unforeseen physiological effects

Discovery of novel clinically efficacious and well tolerated E3 ligase inhibitors would represent a major leap forward in the targeting of the UPS for drug discovery

E1, E2 and E3 as Drug Targets - Summary

E1 ubiquitin activating enzyme

Due to their requirement for ATP, E1 appears to be an attractive drug target based on the previous development of ATP-competitive inhibitors as kinase therapeutics. Conversely, as with proteasome inhibition through Velcade, E1 ubiquitin activating enzymes as a drug target area may suffer from a lack of specificity through its hierarchical position in the UPS. However this does not rule out E1 inhibitors as potential therapeutics if a suitable therapeutic window can be achieved

E2 ubiquitin conjugating enzymes

E2 ubiquitin conjugating enzymes have the potential to provide targets that have enhanced selectivity over E1 ubiquitin activating enzyme and proteasome targets but significant progress has yet to appear

E3 ubiquitin ligases

Whilst, in theory, inhibitors of E3 ligases should be highly specific drugs with few side effects they have proven to be difficult drug targets thus far. Discovery of novel clinically efficacious and well tolerated E3 ligase inhibitors would represent a major leap forward in the targeting of the UPS for drug discovery

Drug Discovery in the UPS

Deubiquitinating Enzymes (DUBs) as Drug Targets

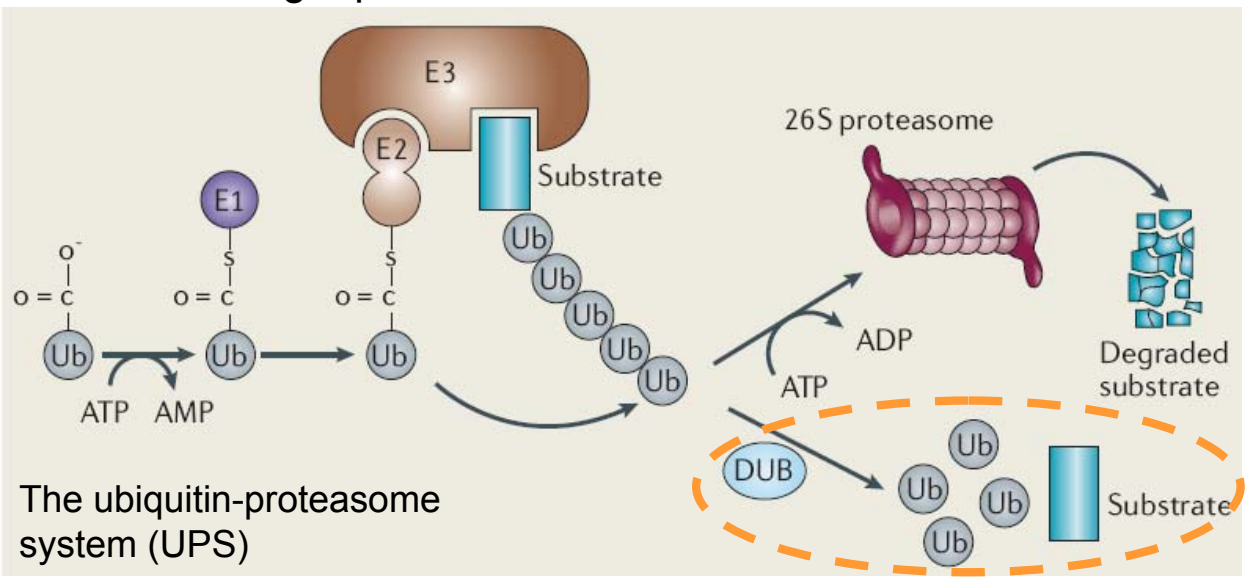
Deubiquitinating Enzymes (DUBs)

Deubiquitinating enzymes (DUBs) mediate the specific removal and processing of ubiquitin from substrate proteins as shown below

As such, they play an opposing role to the E3 ubiquitin ligases, addressing the balance of ubiquitination versus deubiquitination of protein substrates

Some DUBs can also remove ubiquitin-like (UBL) molecules from protein substrates, although proteases selective for each of the UBLs are found as well

- For example, the SENP family of cysteine proteases specifically remove SUMO molecules from target proteins



The ubiquitin-proteasome system (UPS)

DUBs Belong to the Protease Superfamily of Enzymes

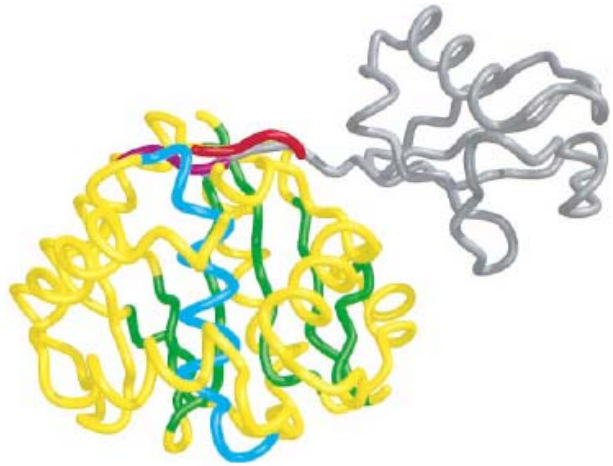
DUBs belong to the protease superfamily of enzymes, which have been targeted successfully by the Pharma industry, and more specifically to the cysteine- or metallo-class of proteases

Cysteine protease DUBs

- The mechanism of action relies on the thiol group of a cysteine within the active site
- During catalysis, the cysteine performs a nucleophilic attack on the carbonyl of the scissile peptide bond e.g. between the protein substrate and ubiquitin
- This results in the release of the protein substrate and the formation of a covalent intermediate with the ubiquitin moiety, which releases free DUB and ubiquitin upon reaction with water

Metalloprotease DUBs

- The mechanism of action generally relies on the use of a Zn^{2+} bound polarized water molecule
- This generates a non-covalent intermediate with the protein substrate which is further broken down by proton transfer from a water molecule to release the free components



An example of DUB-ubiquitin binding. The crystal structure of the DUB is shown with β-strands in green, helix 4 in cyan and other structure in yellow. The structure of ubiquitin is shown in grey

Because of their mechanism of action, many consider DUBs to be more amenable to small molecule drug discovery than other targets within the UPS

Structural Analysis Leads to Classification of DUBs

Cysteine protease DUBs can be further divided into 4 subclasses based on their ubiquitin-protease domains:

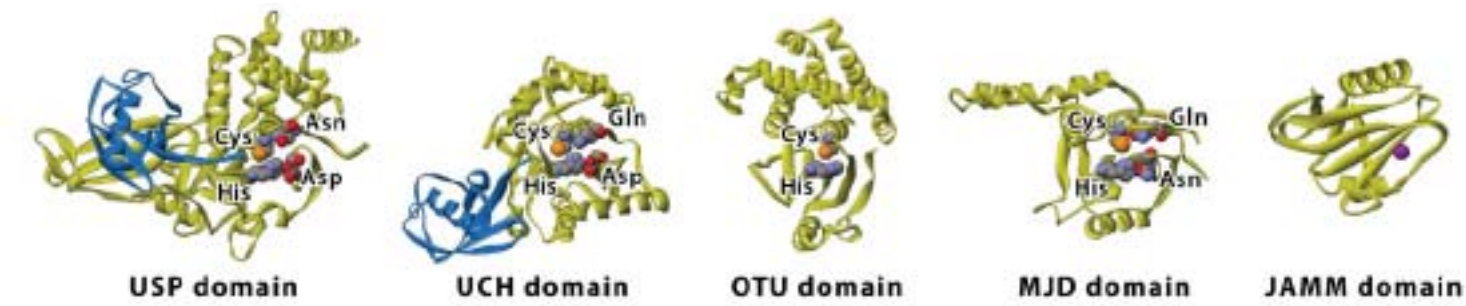
- Ubiquitin-specific protease (USP)
- Ubiquitin C-terminal hydrolase (UCH)
- Otubain protease (OTU)
- Machado-Joseph disease protease (MJD)

Metalloprotease DUBs contain a consensus ubiquitin protease domain, JAMM (JAB1/MPN/Mov34 metalloenzyme)

Analysis of the human genome has identified 95 putative DUBs, based on the presence of one of the above protease domains, although additional DUB-like molecules may exist

Given their opposing roles with E3 ligases, it is surprising that only 95 DUBs appear to exist compared with ~600 E3 ligases identified thus far. This suggests that one DUB is responsible for deubiquitinating multiple protein substrates, thereby conferring reduced specificity from a drug targeting perspective. Alternatively, unidentified cofactors may work with DUBs to increase their specificity profiles

3D structures of DUB catalytic domains from all subclasses, some of them in complex with ubiquitin derivatives, have been resolved (see below)



Possible Mechanisms for Inhibiting DUBs

Accumulating evidence indicates that most DUBs regulate a limited number of proteins and pathways, suggesting that they target specific substrates

DUB specificity can refer to either:

- The ubiquitin or UBL moiety itself (substrate specificity), for example:
  - The protein product of the Cylindromatosis tumour-suppressor gene (CYLD), a DUB involved in inhibiting NF-κB signalling is specific for non-K48-linked ubiquitin chains. The specificity is linked with the catalytic domain of CYLD, suggesting that the architecture of the enzymatic cleft contributes to its specificity
  - Preliminary evidence suggests that domains outside the catalytic domain may also influence substrate specificity, for example the ubiquitin interaction motif in USP15 was recently shown to be required for cleavage of branched, but not linear, ubiquitin polymers
- The target protein to which the moiety is conjugated (target specificity), for example:
  - Many RING finger E3 ligases are auto-ubiquitinated and require continual association with relevant DUBs to prevent their degradation. The E3 ligase NRDP1 stimulates its own turnover as well as a number of cellular targets. The DUB USP8 associates with NRDP1 resulting in its deubiquitination and stabilization. Similarly, the interaction of USP7 with HDM2 and USP15 with Rbx1 results in the stabilization of these E3 ligases

Thus, DUBs may be targeted via multiple different sites of interaction on both the ubiquitin substrate and/or the target protein, as well as via the DUB catalytic domain, and additional allosteric binding sites on the DUB itself

DUBs and Physiological Processes/Disease

From a drug targeting perspective, DUBs have been linked with a variety of cellular processes yet current knowledge remains relatively limited. Some associations of DUBs and their effects are shown below:

DUBs and Proteasome function

Deubiquitination of proteins arriving at the proteasome allows for recycling of ubiquitin and protein degradation. DUBs associated with the 19S proteasome include: the JAMM protease POH1, UCH-L5, and USP14, but only POH1 deletion results in loss of proteasomal function. The other DUBs are likely to ensure the rescue of proteins that have been mistakenly ubiquitinated

DUBs and Oncology

The DUB USP7 associates with HDM2, an E3 ligase critical for regulating p53 turnover and thereby inhibits degradation of both HDM2 and p53

DUBs and Endocytosis

Following ligand binding, receptor tyrosine kinases, such as EGFR are monoubiquitinated, triggering endocytotic internalisation. This results in either recycling back to the membrane or transport to lysosomes for degradation. USP8 is known to deubiquitinate EGFR and inhibit its internalisation

DUBs and Neurodegenerative disease

Mutations in the DUB UCH-L1 that reduce its activity have been described in Parkinson's disease (PD) and a polymorphism in this gene has been linked to reduced PD risk. Instability of a CAG nucleotide repeat in the Ataxin-3 (an MJD DUB) gene leads to a hereditary neurological condition known as Machado-Joseph disease

DUBs as Drug Targets

DUBs, via removal of ubiquitin, play a key role in the reversible nature of the UPS, implicating their appeal as potential drug targets

- In addition, their mechanism of action as proteases further exemplifies them as druggable targets

As a caveat, DUBs are likely to deubiquitinate multiple protein substrates and therefore appear to be less specific that E3 ligases as potential drug targets

- Furthermore, whilst certain DUBs have been putatively linked with some disease states, understanding these links from a potential drug discovery perspective remains embryonic

Elucidation of the combination of E3s and DUBs as specific protein targets in key regulatory pathways would lead to screens for compounds that inhibit either an E3 or DUB respectively

DUB enzymes are considered as the next set of enzymes to target in the UPS, with a number of groups initiating new validation programs, although this remains at an early stage

Competition

The Competitive Space

From a competitive perspective, drug discovery within the UPS is at an early stage

- This is reinforced by the lack of compounds that have reached clinical trials

As in other early stage areas Foresighted, a large proportion of relevant research lies in the halls of academia at present

- This research is primarily aimed at understanding mechanisms of ubiquitin transfer with a large structural biology component, although preliminary drug discovery programmes are in progress

Due to the nature of the targets within the UPS, many comparisons have been made to the kinase class of drug discovery targets

- Kinases are currently one of the top targets for the Pharma industry with up to 30% of Research budgets being spent on this target class
- Kinase drug discovery is seen by many as a forerunner to ubiquitin drug discovery
- Estimates have suggested that the knowledge base surrounding targets within the UPS are between ten and fifteen years behind those in the kinase field

This suggests that whilst drug discovery in the UPS is currently at a very early stage, the potential for future development is substantial

Commercial Deals in the Ubiquitin Space

Due to the early stage of the area, relatively few deals have been completed or disclosed. Deals comprise of discovery alliances which mirror the undeveloped state of drug discovery and lack of lead compounds. Some relevant deals include:

Rigel and Merck

In November 2004, the companies entered into a collaboration to investigate ubiquitin ligases to find treatments for cancer. Progress in this collaboration triggered a $1M milestone payment from Merck in June 2006

Rigel and Daiichi Pharmaceuticals Co.

Collaborating to discover and develop inhibitors of a specific ubiquitin ligase target involved in cancer (August 2002). Rigel received two milestone payments from Daiichi for the successful delivery of two apparently potent and selective ligase inhibitors (May 2005)

Rigel and Janssen (Johnson & Johnson)

Collaborating on the development of small-molecule cell cycle modulators which inhibit UHRF1 ubiquitin ligase, for the treatment of cancer (Nov 2005)

Proteologics and Teva

In May 2005, Proteologics announced the signing of a Feasibility Study Agreement with Teva Pharmaceutical Industries Ltd involving novel drugs targeting ubiquitin ligases for cancer therapy

Cytogen and Celgene

In January 2003 AxCell Biosciences, a subsidiary of Cytogen Corporation, signed a research collaboration with Celgene Corporation focused on specific ubiquitin ligases and their role in cancer and inflammation

It is likely that as more targets within the UPS are validated, many companies observing the area will seek to collaborate or acquire. This further substantiates this area as attractive from an ITI point of view

Summary of Industrial Competition

The following table highlights some drug discovery initiatives within the biotech and pharma industries

No drug candidates targeting E1, E2, E3, UBL or DUB-related sites have been reported to reach a clinical stage to date

Company	Targets	Disease	Stage
Rigel	E3	Oncology, Inflammation, Virology, Metabolism	Preclinical
Celgene	E3	Oncology	Preclinical
Progenra	E3, DUBs	Unspecified	Discovery
Topotarget	DUBs	Oncology	Discovery
Hybrigenics	E3, DUBs	Oncology	Discovery
Regeneron	E3	Muscle atrophy	Discovery
Genentech	p53, COP1	Oncology	Discovery
Millennium	Proteasome, Nedd8	Multiple diseases	Velcade approved for Multiple myeloma, PI-III for other indications
Proteologics	E3, proteasome	HIV, Influenza, Cancer	Discovery
Nereus	Proteasome	Solid tumours, Lymphomas and Multiple myeloma	Phase I
Hoffman-LaRoche	p53, mdm2	Oncology	Preclinical
Meso Scale Discovery	E3	Oncology	Discovery
Cyclacel	p53, mdm2	Oncology	Preclinical
Teva	E3	Oncology	Discovery
Novartis	E3	Unspecified	Discovery
Roche	E3	Unspecified	Discovery
Merck	E3	Oncology	Preclinical

Industrial Competition – Further Details

Celgene is developing a number of small molecule modulators of potentially proprietary E3 ligases for the treatment of cancer and inflammation. In addition, Celgene is building a leading intellectual property estate in the emerging field of ubiquitin ligase-biogenetic activities and mediated protein turnover that includes thirty-eight potentially proprietary ligase targets

TopoTarget is developing inhibitors of VDU1, for the treatment of cancer. VDU1 is a member of the DUB family of enzymes, involved in hypoxia-responsive transcription and angiogenesis. The programme is in early development

Proteologics focuses primarily on discovering inhibitors for specific E3 ligases and their interacting proteins. Proteologics has four discovery programs backed by VC funding and collaborations with industrial and academic institutions. The company is identifying novel, broad-spectrum antiviral drugs to provide more effective treatment against drug-resistant HIV, a variety of influenza strains and other viruses. In parallel, the company is developing therapeutic agents to interfere at specific ubiquitin pathway sites relevant to a number of cancer tumour types. Two cancer projects are being developed in collaboration with Teva Pharmaceutical Industries Ltd. Drs. Avram Hershko and Aaron Ciechanover, 2004 Nobel Prize in Chemistry laureates for the discovery of the ubiquitin system, lead their scientific advisory board.

Industrial Competition – Further Details

Rigel is investigating and characterizing the ubiquitin ligase system for the discovery and development of potential new therapeutics. The company has initiated one of the industry's broadest efforts in working on the development of numerous ligase targets in oncology, inflammation, virology and metabolism. Rigel was one of the first companies to discover potent and highly selective small molecule inhibitors of ubiquitin E3 ligases. The infrastructure has been established through collaborations with Merck and Daiichi. Rigel has an extensive patent portfolio covering the ligase area.

Genentech have published on the role of the E3 ligase COP1 as a critical negative regulator of p53 and hence its implication in oncogenic control, as well as on the E3 ligases involved in NFκB activation

Roche may be one of the more advanced Pharma companies in the ubiquitin area, based on its 2004 publication in Science describing small-molecule Mdm2 E3 ligase-p53 binding inhibitors, called nutlins.

Industrial Competition – Further Details

Hybrigenics runs its own drug discovery programs primarily in oncology. The company is employing its yeast two hybrid screening technology to unravel targets in ubiquitin-regulated protein degradation, specifically E3 ligases and DUBs. Hybrigenics is also screening DUBs involved in cell cycle control in the HCT116 colorectal cancer cell line

Nereus Pharmaceuticals is developing a proteasome inhibitor (NPI-0052) for solid tumours, lymphomas and multiple myeloma. NPI-0052 is active against multiple myeloma cells that are resistant to Velcade, steroid therapy and thalidomide. The compound has also shown efficacy in animal models of myeloma, colon, pancreatic and lung cancer when administered orally or intravenously and is in Phase I clinical trials.

Progenra have a focus on protein degradation in the cell exemplified by ubiquitin pathway enzymes relevant in Parkinson's disease, cancer, myopathy, arthritis, and osteoporosis. The company is developing a range of gene libraries, technologies and research tools towards all of the ubiquitin-like proteins (including SUMO, ISG15, Nedd 8), several E3 ligases, DUBS (including Ubp43, Den1, Senp2, USP2a). Progenra is also developing antibodies to several ubiquitin-like proteins (in collaboration with Rockland Immunochemicals, Inc)

Industrial Competition – Further Details

Regeneron is using its VelocImmune technology platform, designed to produce fully human monoclonal antibodies, to build its next generation of clinical development candidates. Regeneron is interested in E3 ligases but have not yet announced a clinical candidate. Regeneron is focusing on muscle atrophy.

Following on from the launch of VELCADE® (bortezomib) for Multiple Myeloma, Millennium have a range of ongoing clinical trials for VELCADE including: Phase III, open label study for previously untreated multiple myeloma, Phase III study in newly diagnosed Multiple myeloma patients, Phase III study in the transplant setting, Phase III clinical trial in non-Hodgkin's lymphoma. Additionally, a number of Phase I and II clinical trials for various tumour types including lung, breast, prostate and ovarian cancers and non-Hodgkin's lymphoma are underway.

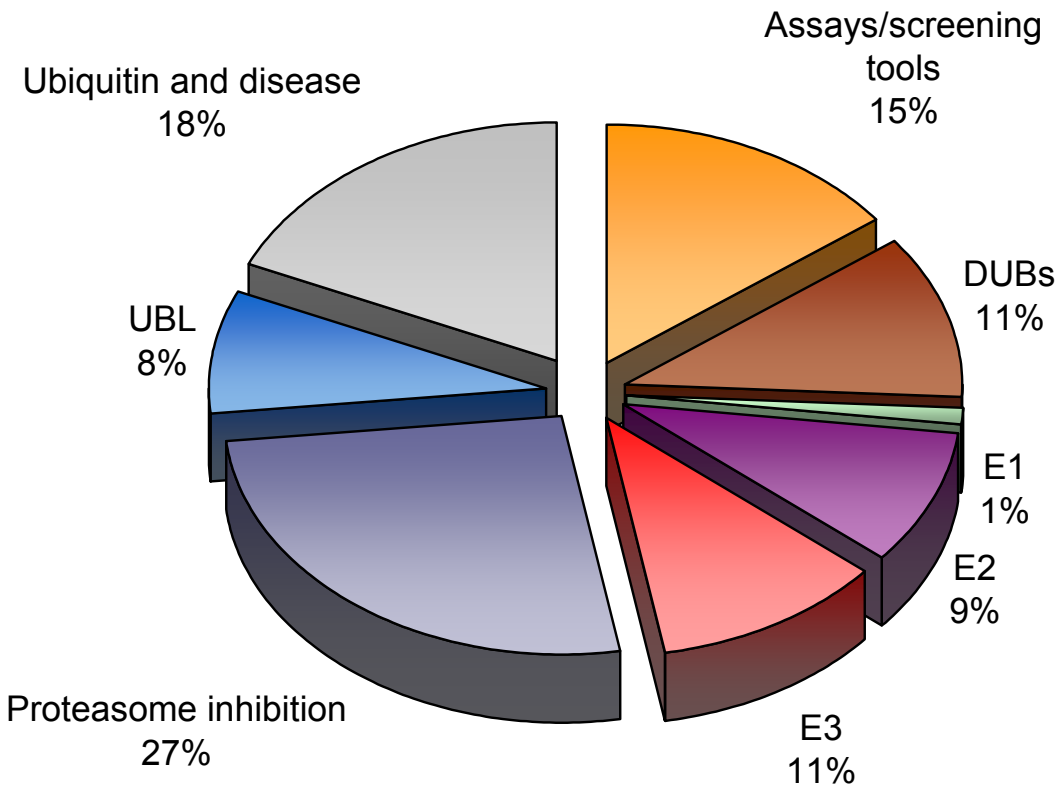
The Genomics Institute of the Novartis Research Foundation has recently created an ubiquitination group within its Department of Cancer and Cell Biology.

IP Landscape

Overview of UPS-Related Patenting Activity

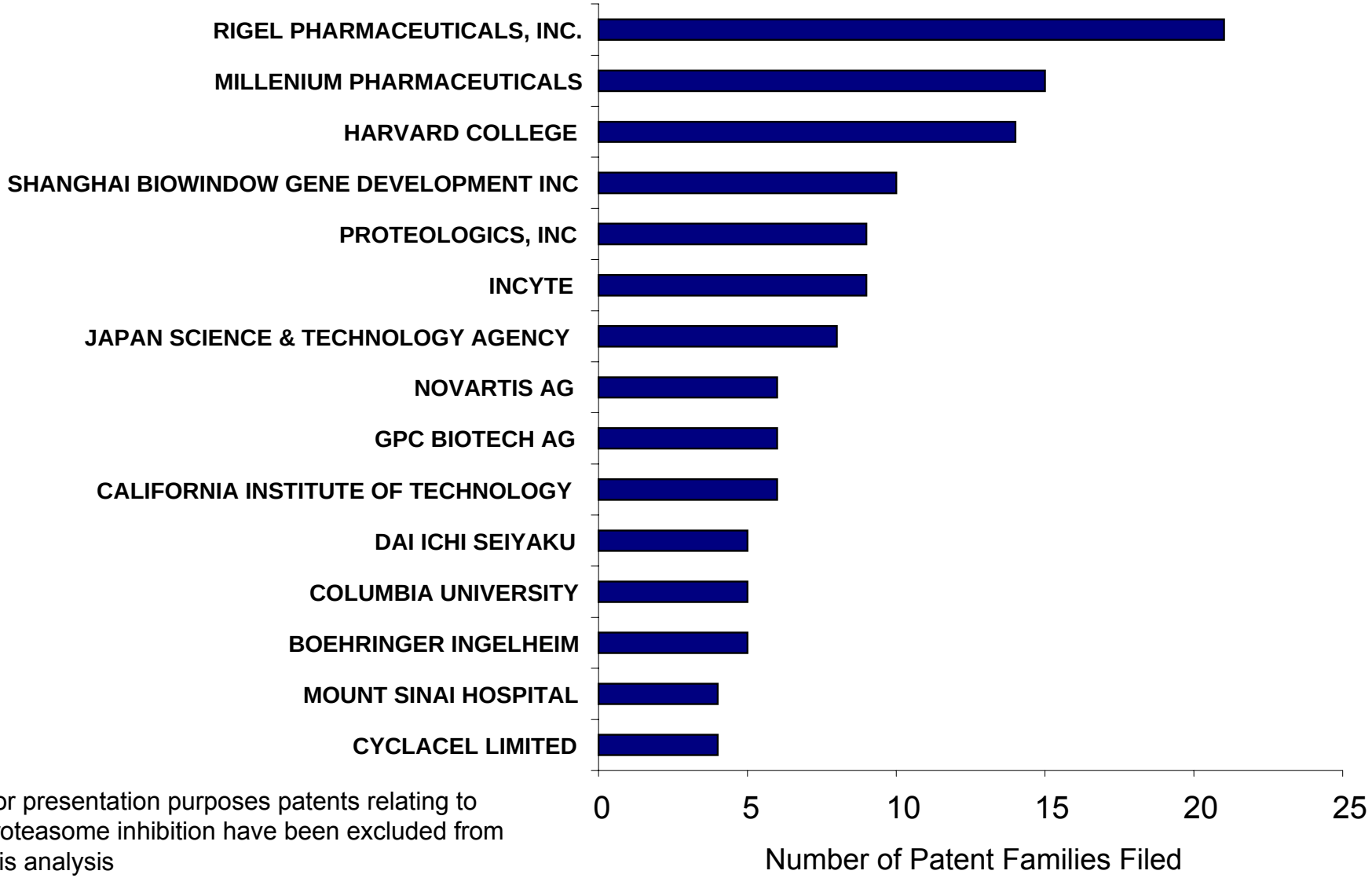
Our initial analysis of patenting activity related to the UPS has revealed:

- A relatively low level of patent activity despite substantial growth since 2000. For example patent activity per annum within the kinase field is approximately 50-fold greater than in the UPS
- The majority of the patents relate, in part, to proteasome inhibition as a consequence of the clinical development of Velcade
- The numbers of patents in each of the other categories appear to be comparable, with the exception of E1 activating enzymes which are significantly lower



UPS-related patenting activity by defined categories

Organisational Patenting Activity



For presentation purposes patents relating to proteasome inhibition have been excluded from this analysis

UPS Patent Map

Keywords within the identified set of patents and patent applications related to the UPS are grouped into topics to produce a map

Collections of patents which share common elements are geographically close together whilst collections with less similarity are further apart. The map also highlights disease areas (Autoimmune diseases, Alzheimer's disease and cancer) and/or other signalling pathways (kinases) that are linked with patenting within the UPS

The patent landscape is therefore displayed as a series of mountain tops and valleys with the higher peaks such as proteasome inhibition representing the larger patent collections.

Drug Discovery in the UPS

Drivers and Challenges

Drivers and Challenges

So far we have discussed components of the UPS, potential drug discovery targets within the UPS and their implication in disease and also described the competitive and intellectual property landscape……

…… the aim of this section is to summarise the main drivers and challenges which are currently influencing the degree and scale of drug discovery in this area

Drivers for Drug Discovery in the UPS (I)

The UPS has been implicated in multiple disease states including cancer, inflammatory disorders and neurodegenerative disorders. Current treatments for these diseases are suboptimal and drug discovery in the UPS may provide therapeutic solutions

- Discovery of a novel validated target and concomitant lead series with therapeutic advantages over current best in class drugs would precipitate considerable commercial attention
- Discovery of a novel first in class therapeutic would likely lead to a cascade of follow on clinical candidates thereby adding additional commercial value to support future drug discovery

The approval of Velcade has provided proof of concept and validated the UPS as a target for modulating disease

- Despite this, Velcade is not an ideal drug due to multiple undesirable side effects which are attributed to its lack of specificity via proteasome inhibition. This has opened the door for follow on proteasome inhibitors and novel classes of drugs targeting upstream components of the UPS which may have increased specificity and decreased mechanism-based liabilities

Drivers for Drug Discovery in the UPS (2)

Similarities have been drawn between drug discovery in the UPS and kinase drug discovery, which is currently one of the top molecular targets, accounting for up to 30% of total R&D spend within Pharma

- This momentum may fast track drug discovery in the UPS which is estimated to be 10-15 years behind the development of kinase drug targets as therapeutics
- Additionally, the level of interaction between phosphorylation and ubiquitin pathways and *vice versa* is an emerging area that is likely to add further strategic options in drug discovery and development
- Many academic and industrial luminaries with a focus on phosphorylation are starting to work in the ubiquitin field, highlighting the important role that the UPS may play in their research going forward

Most drug targets within the ubiquitin pathway appear amenable to X-ray crystallography

- Many groups have successfully resolved crystal structures of both individual molecules and complexes within the UPS, although further structural elucidation will drive progress
- This will facilitate *in silico* screening and rational drug discovery based approaches that may speed up hit discovery in this area

The highly conserved nature of ubiquitin may facilitate rapid translation of knowledge gained across species

- For example, understanding the components and roles of the UPS in relatively simple systems such as yeast and plants may result in the discovery of human orthologs that further aid our understanding and potential exploitation within the human UPS

Challenges for Drug Discovery in the UPS (I)

Aside from proteasomal inhibition through Velcade, all other targets within the UPS have not been validated thus far, therefore increasing the risk profile of drug discovery in this area

- Drug discovery within the UPS will require the modulation of a novel set of molecular targets
- The UPS is emerging as a much more complex system than comparable drug target areas, such as kinases
- Nuances in the exact mechanisms involved along the UPS pathway are still being challenged and the standard E1-E2-E3 paradigm may not hold true for all UPS-like pathways and their components

Linking the growing knowledge of the UPS and the range of potential drug targets to disease is a significant challenge

- Is the target (over/under) expressed in relevant human diseased tissue?
- Is target expression a driver or a consequence of the disease state?
- Is the target the correct intervention point to individually halt or reverse disease?

Despite the growing interest in the area from academics, research institutes and commercial companies, there has been relatively little evidence of significant drug discovery progress to date

- This suggests that drug discovery in the UPS will be a long term effort
- Mobilisation of a diverse range of scientific disciplines and collaborators will assist progress

Challenges for Drug Discovery in the UPS (2)

Choosing the appropriate UPS target for drug discovery and development is a complex decision

- This involves a trade-off between the stage of modulation in the UPS, such as E3 (high specificity) and proteasome inhibition (low specificity)
- How druggable is the target? The complex array of protein-protein interactions in the UPS makes small molecule inhibition problematic
- Biological inhibition, using peptides, antibodies in various forms or RNAi, may fast track target validation, although these offer additional challenges with respect to formulation, delivery and cost

Exploiting the UPS for drug discovery may have unanticipated consequences

- The likelihood for multiple downstream effects through ubiquitination of multiple protein substrates and signalling pathway crosstalk, increases the chances of detrimental side effects making some UPS targets less attractive than others
- The modulation of one pathway may not lead to the desired therapeutic results due to the induction of compensatory pathways

UPS Related Research in Scotland

UPS Related Research in Scotland

The majority of the research related to ubiquitin signalling in Scotland is being carried out by a number of Universities and Research Institutes, including:

- The School of Life Sciences, University of Dundee
- The CRUK Cell Signalling Unit, University of Edinburgh
- The Institute of Biomedical and Life Sciences and The MRC Virology Unit, University of Glasgow
- The Beatson Institute for Cancer Research, Glasgow
- The Institute of Medical Sciences, University of Aberdeen
- Cyclacel plc

Conclusions

Conclusions (1)

- The UPS provides a rich source of emerging drug targets, yet it is an unexploited and largely uncharted area for drug discovery
- The approval of the proteasome inhibitor, Velcade, for Multiple myeloma has proven the principle that the UPS in general, is a valid system for therapeutic intervention
- In recent years, the UPS has been implicated with a growing list of key cellular functions related to disease, aside from its original association with protein degradation via the proteasome
- Broader roles of the UPS in disease is driving increased academic and industrial attention although drug discovery within the area remains at a very early stage. Progress may be fast-tracked through the momentum gained from kinase drug discovery
- Discovery of a novel validated target and concomitant lead series with therapeutic advantages over current best in class drugs would precipitate considerable commercial attention
- Discovery of a novel first in class therapeutic would likely lead to a cascade of follow on clinical candidates thereby adding additional commercial value to support future drug discovery

Conclusions (2)

- Whilst, in theory, inhibitors of E3 ligases should be highly specific drugs with few side effects they have proven to be difficult drug targets thus far
- DUB enzymes are considered as the next set of enzymes to target in the UPS. Due to their function as cysteine- or metallo-proteases, DUBs are considered to be more amenable to small molecule drug discovery. As a caveat, DUBs are likely to deubiquitinate multiple protein substrates and therefore appear to be less specific that E3 ligases as potential drug targets
- Many of the UPS drug targets are not obviously amenable to small molecule inhibition due to multiple complex protein-protein interactions
- Any decision on the appropriate drug target for exploitation will involve a trade-off between disease linkage, selectivity, sensitivity, and druggability of the target together with the level and quality of market competition
- From an initial scan of Scottish competencies in this area, it is clear that a number of parties possess some key building blocks which would be essential for impacting drug discovery on this emerging drug target area

Next Steps

What Happens Next?

To further determine where specific opportunities lie within Ubiquitin Signalling as an Emerging Drug Target area, we would very much welcome dialogue with our Members

To arrange a discussion, please contact ITI Life Sciences at:

foresighting@itilifesciences.com

Tel. +44 (0)1382 568060

For more information on ITI Life Sciences, please visit

http://www.itilifesciences.com/