Networks regulating ubiquitin and ubiquitin-like proteins promise new therapeutic targets

Deborah J Marsh
Hormones and Cancer Group, Kolling Institute of Medical Research, Royal North Shore Hospital, University of Sydney, St Leonards, Sydney, New South Wales 2065, Australia

The 2004 Nobel Prize in Chemistry jointly awarded to Aaron Ciechanover, Avram Hershko and Irwin Rose celebrated the significance of their discovery that proteins were ‘labelled for destruction’ through recognition of a controlled molecular ‘kiss of death’, the addition of a chain of the 8.5 kDa (76 amino acid) protein ubiquitin. Degradation via the ubiquitin-proteasome pathway involves polyubiquitination of substrate proteins followed by proteolytic degradation by the macromolecular 26S proteasome complex. Key cancer-associated proteins whose levels are tightly controlled by the ubiquitin-proteasome include p53, p27, cyclins and BCL2 family members. The enzymes involved in conjugation and deconjugation of ubiquitin to protein substrates include an activating ATP-dependent ubiquitin enzyme (E1), a ubiquitin-conjugating enzyme (E2), ubiquitin-protein ligases (E3s) that often form multi-component complexes key for substrate recognition, and deubiquitinases (DUBs) that cleave ubiquitin from protein substrates. In humans, there are just a few E1 enzymes, around 40 E2 enzymes, over 500 E3 ligases (most commonly RING and HECT domain E3s) and around 100 DUBs, the majority belonging to the ubiquitin-specific protease (USP) sub-family (Lipkowitz & Weissman 2011, Budhidarmo et al. 2012, Jacq et al. 2013). These enzymes have major regulatory roles in normal cellular processes, both within and independently of the ubiquitin-proteasome, including DNA repair, maintaining genomic stability and transcription. Aberrant expression of a number of DUBs and E3s has been linked to cancer (Lipkowitz & Weissman 2011, Clague et al. 2013). As a consequence, many of these enzymes are generating extensive interest as targets for the treatment of cancer.

In his review in this special issue of Endocrine-Related Cancer on Ubiquitination and Cancer, Johnson 2015 describes how aberrant expression of oncogenes and/or tumour suppressors can disrupt normal cellular processes such as cell cycle progression or apoptosis. Inhibiting proteasomal degradation of proteins that may help to kill cancer cells is a strategy that has led to the development and use of first and now second generation proteasome inhibitors. Bortezomib (Velcade) was, in 2008, the first proteasome inhibitor approved by the US Food and Drug Administration (FDA) for the treatment of multiple myeloma, with the second generation inhibitor Carfilzomib (Kyprolis) approved in 2012 for treatment of the same malignancy (reviewed in Shen et al. (2013)). FDA approval has also been given to use these drugs for the treatment of mantle cell lymphoma. Thus far, these drugs have shown considerable clinical benefit, although not without significant adverse toxicity and issues of acquired resistance. With lessons learnt from the treatment of haematological malignancies, proteasome inhibitors are now being explored for the treatment of solid tumours. Johnson 2015 describes emerging clinical studies of a number of proteasome inhibitors using mono- or combinatorial strategies (Bortezomib, Carfilzomib, MLN9708, Delanzomib, Marizomib and Oprozomib) to treat solid tumours including breast, colorectal, pancreatic, head and neck, hepatocellular and prostate cancers, as well as melanoma, small and non-small cell lung carcinomas and renal cell cancers.

An alternative strategy to inhibiting the proteasome is to move upstream to target the regulatory network of enzymatic reactions directing conjugation and deconjugation of ubiquitin to protein substrates. This approach...
Endocrine-Related Cancer and Pfoh shown to overcome bortezomib resistance (Chauhan models of multiple myeloma where this inhibitor has been small molecule inhibitor P5091, have shown promise in Pre-clinical studies of USP7 inhibitors, including the conceivably be achieved through inhibition of USP7. Restoration of functional WT p53 could therefore proteasomal degradation of this tumour suppressor. thus facilitating polyubiquitination of p53 leading to effect on p53. USP7 deubiquitinates the p53 E3 ligase DUB USP7 is garnering extensive interest, largely due to its number of cancer-associated E3s and DUBs. Inhibiting the development of novel cancer therapeutics.

While the importance of protein ubiquitination was discovered in the context of polyubiquitination, the addition of a single ubiquitin to specific protein substrates, known as monoubiquitination, has also been shown to have critical cellular functions. An example of this is monoubiquitination of the Fanconi Anemia protein FANCD2 in response to DNA damage that promotes homologous recombination repair of DNA double strand breaks (Nakanishi et al. 2005). The review by Cole et al. focusses on another significant example of monoubiquitination, specifically monoubiquitination of histone H2B at lysine 120 (H2Bub1), referred to as a master switch for mammalian gene regulation. Post-translational histone modifications (methylation, acetylation, phosphorylation, ubiquitination etc.) are fundamental in shaping the chromatin landscape, with key E3s referred to as chromatin writers and DUBS as chromatin erasers, all influencing the accessibility of chromatin for the purposes of transcription and DNA repair (Braun & Madhani 2012). H2Bub1 has significant roles in transcription, DNA damage response and stem cell differentiation. Nine DUBs, including USP7 and USP22, have to date been shown to regulate deubiquitination of H2Bub1, with the main E3 ligase being the ring finger complex RNF20/RNF40. The BRCA1/BARD1 complex has also been shown to function as an E3 ligase for H2Bub1. Loss of H2Bub1 leads to closed chromatin, inhibiting access by transcription factors and DNA repair proteins. Global loss of H2Bub1 has been reported in advanced malignancies, including breast, colorectal, lung and parathyroid cancers. The ubiquitin ligation machinery regulating H2Bub1 levels in malignancy is being considered for the development of targeted therapies.

Ubiquitin-like proteins, such as NEDD8-reviewed in this issue by Abidi & Xirodimas (2015), show high homology to ubiquitin and use similar sets of enzymatic processes. Targets of NEDDylation include the family of cullin proteins that are scaffold proteins with roles in regulation of the cell cycle, DNA repair, cytoskeletal dynamics and the hypoxic response. NEDD8 inhibitors are currently being developed and the first-in-class small molecule inhibitor of NEDD8 activating enzyme (NAE) MLN4924 has entered clinical trials for the treatment of adults with non-haematological malignancies. Issues of acquired resistance are being monitored, as well as consideration of combinatorial strategies, including radiation and chemotherapies.

As therapies targeting ubiquitin and ubiquitin-like pathways for solid tumours begin to move into the clinic, they are benefitting from strong past and ongoing discoveries that are elucidating these complex networks and the proteins they regulate, both as part of, and independently from, the proteasome. These discoveries will be key to overcoming the development of resistance and minimising adverse side effects of these new targeted cancer therapies.

Declaration of interest
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