inhibitors of the sodium and potassium transporter protein, called the Na^+/K^+-ATPase, suppress CTC clusters and block metastasis. A drug called digoxin, which inhibits the Na^+/K^+-ATPase, is approved for the treatment of cardiac diseases, and digoxin became the focus for further clinical testing. The Na^+/K^+-ATPase has a key role in generating the ion (electrochemical) gradients across the cell membrane that provide the electrical charge of the cell when it is at rest (the resting potential) or when it is active. This transporter exports three sodium ions out of the cell for every two potassium ions imported[4]. Moreover, the Na^+/K^+-ATPase can act in several signalling pathways and affect the regulation of other ions as well as cellular properties[4,5], making it an interesting and versatile target for anticancer therapy.

In this pilot study of people who have breast cancer with metastasis, Kurzeder and colleagues treated nine individuals daily with a dose of digoxin, which resulted in an average reduction of 2.2 cells per CTC cluster (before treatment, the average number of cells in a cluster was around four). This response to digoxin was associated with lowered expression of genes involved in cell–cell adhesion and cell division (Fig. 1). The treatment was safe, with no treatment-related adverse events reported.

This proof-of-concept study might prompt follow-up studies testing digoxin or other inhibitors of the Na^+/K^+-ATPase. More people will need to be tested to draw firmer conclusions about whether this might provide a cancer treatment option. These studies should also include a control group and assess clinical outcomes such as progression-free survival (survival time without any worsening of disease) or overall survival (survival rates after treatment). Metastatic breast cancer shows high variability in clinical outcomes between individuals. Therefore, an observation based on nine people with cancer is hypothesis-generating rather than fully conclusive.

Although the cluster reduction was statistically significant, it was rather moderate in terms of the number of cells lost per cluster, raising doubts about whether a partial and moderate CTC-cluster dissolution could change the course of disease for people with advanced cancer who already have cancer growths at distant sites (metastases). This cluster dissolution would limit the number of new metastases but not the growth and progression of existing metastases that might drive the clinical disease outcome. Furthermore, digoxin treatment would not affect the clustering of CTCs with blood cells such as neutrophils that also support metastatic progression[6]. Finally, it should be noted that single CTCs predict an unfavourable clinical outcome in breast cancer and some other tumours, as demonstrated in clinical studies on large groups of individuals[7,8], even if the metastatic potential of single CTCs might be lower than that of CTC clusters.

In future clinical studies of the effects of digoxin on CTCs, an earlier intervention in people with less-advanced disease might be better at preventing the formation of metastases. Reports have indicated[9] that blood analyses for CTCs (or circulating cell-free fragments of tumour DNA) can detect cancer cells that remain in the body after treatment (termed minimal residual disease). If CTCs are detected, people have a higher chance of developing a metastasis. However, a challenge is that CTC clusters have rarely been detected in the typical 10-millilitre blood samples obtained from people at an early stage of cancer who don't have detectable metastases. One way to overcome this bottleneck might be to analyse larger volumes of blood, which can lead to improved CTC capture rates[10].

For clinical implementation, it will be crucial to harmonize and standardize the published protocols for detection of CTC clusters. International consortia such as the European Liquid Biopsy Society (www.elbs.eu; based in Hamburg, Germany), which K.P. chairs, are engaged in this important task.

Kurzeder and colleagues' work has highlighted the potential of CTC analyses and manipulation for developing anti-metastatic therapies. Viable CTCs can give rise to distant metastases, and an in-depth analysis of these cells might provide insights into the ability of CTCs to form metastases in people who have cancer.

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Cell biology

Signs of damage that drive protein degradation

Alfred Freeberg & Michael Rapé

Many environmental toxins damage proteins, which then must be removed to avoid dangerous protein aggregation and disease. How cells dispose of chemically modified proteins has been unclear, but a discovery offers some clues. **See p.519**

All organisms are constantly exposed to a barrage of environmental insults that can compromise the integrity of cells and tissues. To preserve cellular function, dedicated signalling pathways can detect and alleviate challenges to proteins and the genome. These protective networks are called stress responses, and they often use a modification termed ubiquitylation to mark a damaged protein for degradation. On page 519, Muhar *et al.*[1] provide insights about how the stress-response machinery can eliminate chemically damaged proteins to ensure continued cell function and survival.

The reaction at the heart of stress responses, ubiquitylation, is best known for its ability to drive protein destruction using degradation machinery called the proteasome[2]. In this manner, ubiquitylation controls cell proliferation, inflammatory signalling, as well as the timely execution of cell-death programmes. In human cells, the substrate specificity of ubiquitylation depends on approximately 600 enzymes known as E3 ligases, which often select targets by detecting short stretches of amino acids referred to as degrons[3]. For example, the E3 ligase APC/C binds to D- or KEN-box degrons in proteins that regulate cell division to establish a sequence of protein-degradation events that orchestrates the complex process of producing identical daughter cells[4]. The recognition of E3-ligase substrates can be modulated by other modifications of the target protein, such as the addition of a phosphate group, which couples ubiquitylation to a variety of preceding signalling events.

When encountering cellular stress, E3 ligases must deal with proteins that have

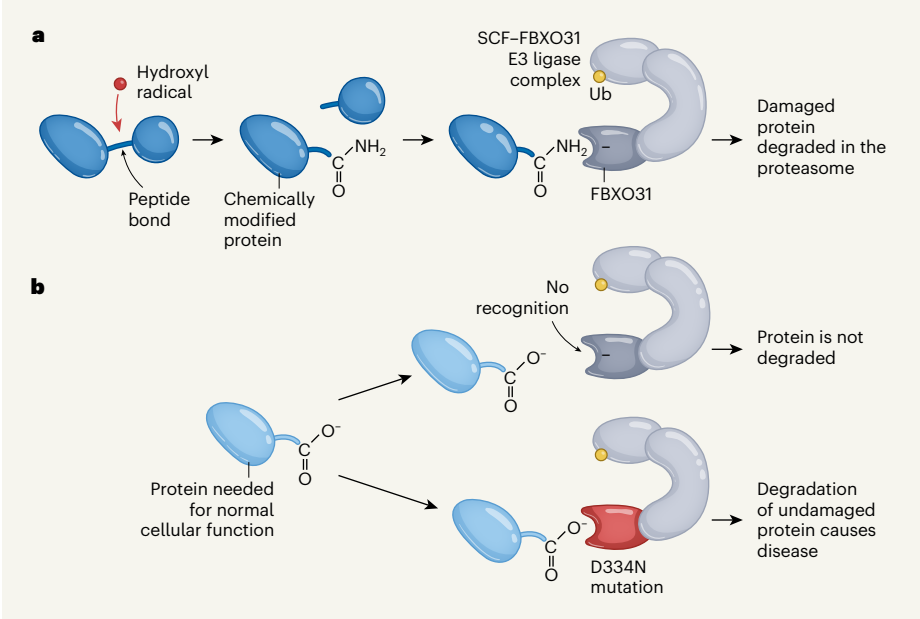

Figure 1 | Identification of a mechanism to eliminate a chemically modified protein. Muhar *et al.*[1] report how a type of enzyme complex called an E3 ligase can recognize damaged proteins that need to be targeted for degradation. **a**, The E3 ligase SCF–FBXO31 recognizes a protein modification that arises on exposure to stress in the form of reactive molecules called hydroxyl radicals. These radicals target peptide bonds and the cleavage of this bond results in a protein with a modified (amidated) carboxy terminus that lacks a negative charge. This group is recognized by the negatively charged binding pocket of the FBXO31 component of the E3 ligase. The recognized protein is tagged with ubiquitin (Ub) proteins that mark it for destruction in degradation machinery called the proteasome. **b**, FBXO31 does not usually recognize the normal carboxy termini of proteins. Proteins with a normal carboxy terminus and certain adjacent amino-acid residues can be recognized by a mutant version of FBXO31 (D334N), which removes a negative charge in the binding pocket. This mutation is associated with cerebral palsy. The recognized normal protein is degraded.

been damaged in many different ways. For example, misfolding can expose multiple hydrophobic regions in the same protein, promoting protein aggregation, and many toxins can chemically modify proteins across their entire sequence. How E3 ligases deal with such diverse substrates, especially those resulting from chemical insults, is still poorly understood. Given that defective ubiquitylation can lead to developmental and degenerative diseases[5], there is a pressing need to reveal the inner workings of this quality-control machinery.

Muhar and colleagues report the development of a method using the gene-editing tool CRISPR to identify E3 ligases that target chemically modified proteins. This system enabled the authors to discover that the E3 ligase SCF–FBXO31, which is a complex composed of a catalytic module (comprising the proteins SKP1, CUL1 and RBX1) and a substrate-specificity factor called FBXO31, recognizes truncated proteins with a particular type of modification — an amidated carboxy terminus. These proteins are also referred to as C-terminal amide-bearing proteins (CTAPs).

CTAPs emerge from cleavage of the protein 'backbone' during oxidative stress (Fig. 1). This cleavage involves the 'attack' of molecules called hydroxyl radicals on the peptide bond, which results in protein fragments with amidated C termini that lack a negative charge. This reaction can occur at many positions across the protein sequence and therefore creates diverse fragments that need to be cleaned up by an enzyme that combines specificity for amidated C termini with a flexibility to recognize proteins with different amino-acid compositions.

SCF–FBXO31 shows such specificity. It binds amidated C termini with high (nanomolar) affinity, but it neither recognizes the normal C termini of unmodified proteins nor does it detect amides in the side chains of the asparagine or glutamine amino-acid residues. Indeed, the identity of the amino acid at the new C terminus of a cleaved protein has only a minor effect on recognition of a damaged protein by SCF–FBXO31, enabling this E3 ligase to target CTAPs that arise from many cleaved proteins. SCF–FBXO31 establishes its selectivity by avoiding the negatively charged C termini of functional proteins and by recognizing additional, still poorly understood, structures using the deep substrate-binding pocket of FBXO31. Reflecting the ability to ubiquitylate CTAPs without much sequence preference, surveys of cellular targets of SCF–FBXO31 reveal that it binds to many different proteins when cells experience oxidative stress that results in the formation of CTAPs.

FBXO31 loss can cause intellectual disability.

From the archive

A skull provides key evidence about human evolution, and early plans for a tunnel to connect England and France.

100 years ago

The discovery of fossil remains of a "man ape" in South Africa raises many points of great interest for those who are studying the evolution of man and of man-like apes. No doubt when Prof. Dart publishes his full monograph of his discovery, he will settle many points which are now left open, but from the facts he has given us, and particularly from the accurate drawing of the endocranial cast and skull in profile, it is even now possible for an onlooker to assess the importance of his discovery. I found it easy to enlarge the profile drawing ... to natural size and to compare it with corresponding drawings of the skulls of children and of young apes. When this is done, the peculiarities of Australopithecus become very manifest ... Prof. Dart recognises the many points of similarity which link Australopithecus to the ... chimpanzee and gorilla ... and yet in certain points it differs from both, particularly in the small size of its jaws ... The most interesting, and perhaps significant, distinctive features are presented by the natural endocranial cast. They may possibly justify the claim that Australopithecus has really advanced a stage further in the direction of the human status than any other ape ... When fuller information regarding the brain is forthcoming ... I for one shall be quite prepared to admit that an ape has been found the brain of which points the way to the emergence of the distinctive brain and mind of mankind. Africa will then have purveyed one more surprise — but only a real surprise to those who do not know their Charles Darwin.

From *Nature* 14 February 1925

150 years ago

At the last *soirée* of the Paris Observatory M. Dupuy de Lome explained his ferry-boat intended to carry railway trains between England and France. M. de Lesseps also delivered a lecture on the tunnel which it is proposed to bore from Calais to Dover.

From *Nature* 11 February 1875

A mutation in FBXO31 that replaces an aspartate with an asparagine residue (a D334N mutation), gives rise to a form of cerebral palsy, hallmarks of which include damage to the cortical region of the brain, muscle stiffness and spasms[6]. Muhar and colleagues report that the D334N variant of FBXO31 no longer recognizes CTAPs. Further effects must contribute to the dominant nature of this mutation — only one of the two copies of the gene needs to be mutated to cause the disease. Indeed, the D334N mutation switches the substrate specificity of SCF–FBXO31 from CTAPs to recognizing proteins with other degrons at their C termini (Fig. 1). This mutation therefore 'rewires' the substrate specificity of an E3 ligase to induce aberrant degradation of proteins that are required for normal cellular function.

Muhar and colleagues' work has identified machinery that protects cells against defective proteins that emerge in response to environmental toxins and oxidative stress. It raises the possibility that more of the approximately 600 human E3 ligases sense chemical modifications, an area of stress-response biology that has not received much attention. How CTAPs emerge under physiological conditions, and why they must be degraded, requires further investigation. Cleavage of proteins probably results in partial unfolding or defective interactions with their normal partners, suggesting that the SCF–FBXO31 targets might also be picked up by other quality-control systems. It will be interesting to examine how SCF–FBXO31 works with other ubiquitylation enzymes that counteract toxic protein species that arise from misfolding or aggregation.

The striking specificity of SCF–FBXO31 is reminiscent of that of another E3 ligase, CUL4–CRBN, which detects a similar type of C-terminal modification called a cyclic imide. These C termini arise from toxin-induced cleavage of a protein at asparagine or glutamine residues[7]. Small molecules, including the drug thalidomide, mimic a cyclic-imide degron and bind CUL4–CRBN in a manner that increases the affinity of this E3 ligase for substrates that it wouldn't normally target (neosubstrates)[8,9], a capacity that has been instrumental in the development of targeted protein degradation as a therapeutic approach[10,11]. It is tempting to speculate that FBXO31's ability to detect amidated C termini allows for similar mimicry of an E3-ligase recognition element by small molecules, providing a starting point to identify compounds that recruit disease-causing proteins to SCF–FBXO31 for degradation. Muhar and colleagues' work therefore not only provides a road map for how to discover quality-control networks that counteract chemical stress, but also points to cellular factors that might be hijacked by small molecules to develop new therapeutic agents.

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