Cancer

Path to destruction for a cell-division regulator

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The molecular mechanism governing the destruction of key cell-cycle proteins, D-type cyclins, has been elucidated. This mechanism might underlie the lack of response of some human tumours to an inhibitor treatment. **See p.789, p.794 & p.799**

Proteins called D-type cyclins (cyclin D1, D2 and D3) are key components of the core cell-cycle engine, which drives cellular division. Simoneschi *et al.*[1] (page 789), Chaikovsky *et al.*[2] (page 794) and Maiani *et al.*[3] (page 799) now provide the long-sought answer to how D-type cyclins are normally degraded.

During cell division, D-type cyclins bind to and activate their enzymatic partners, termed cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6). These cyclin–CDK4/6 kinases add phosphate groups to the tumour-suppressor proteins RB1, RBL1 and RBL2, and this consequently propels cell division (Fig. 1). The uncontrolled activation of cyclin D–CDK4/6 kinases is a driving force in the development of many types of cancer[4].

Interest in cyclin D–CDK4/6 biology has grown lately as small-molecule inhibitors of CDK4/6 kinases have entered the clinic. Remarkable results from clinical trials of these compounds demonstrate their ability to extend the survival times of people with breast cancer. The CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib are approved for the treatment of advanced breast cancers. Moreover, these drugs are being tested in several hundred clinical trials for many different types of cancer[4,5].

D-type cyclins have been intensively studied since their discovery in the 1990s, but how they are degraded during the cell cycle has remained controversial. The phosphorylation of the carboxy-terminal region of D-cyclins triggers the destruction of these proteins by a degradation pathway called the ubiquitin–proteasome system[6]. In this system, a cascade of activity by ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin-ligase (E3) enzymes attaches chains of several molecules of a small protein, ubiquitin, to target proteins, by a process called ubiquitylation. These ubiquitylated proteins are then destined for degradation in a protein complex called the proteasome.

The largest family of E3s are cullin–RING ligases (CRLs). CRLs consist of a cullin protein, a RING protein (which recruits an E2), an adaptor protein and one of the many different substrate-receptor proteins responsible for recruiting the target protein to the E3 complex[7–9]. Several substrate receptors for an E3 called CRL1 have been implicated in the degradation of cyclin D1, whereas others were postulated to target cyclins D2 and D3 for proteasomal destruction. In addition, cyclin D1 was shown to be ubiquitylated by the anaphase-promoting complex, an E3 complex that targets several cell-cycle proteins[9]. In contrast to those models, other research[10] demonstrated that the level and stability of cyclin D1 was unaffected by depletion of these proteins, indicating that some other E3 regulates the degradation of cyclin D1.

The papers by Simoneschi, Chaikovsky, Maiani and their respective colleagues report that three D-type cyclins are ubiquitylated and targeted for proteasomal degradation by the E3 termed CRL4, which uses the protein AMBRA1 as its substrate receptor. It was already known that AMBRA1 has a key role in regulating autophagy, a process which cells degrade damaged organelles or protein aggregates[11,12]. AMBRA1 had also been identified as a substrate receptor of E3s, including CRL4 (refs 7, 13). Through a beautiful series of experiments, using techniques from fields including cell biology, molecular biology and developmental genetics to provide insights in both mice and humans, Simoneschi, Chaikovsky and Maiani *et al.* demonstrate that AMBRA1 depletion in normal cells and cancer cells, as well as in developing mouse embryos, leads to a rise in the level of D-type cyclins. This results in greater phosphorylation of RB1 and more cell proliferation than occurs in cells that have normal amounts of AMBRA1.

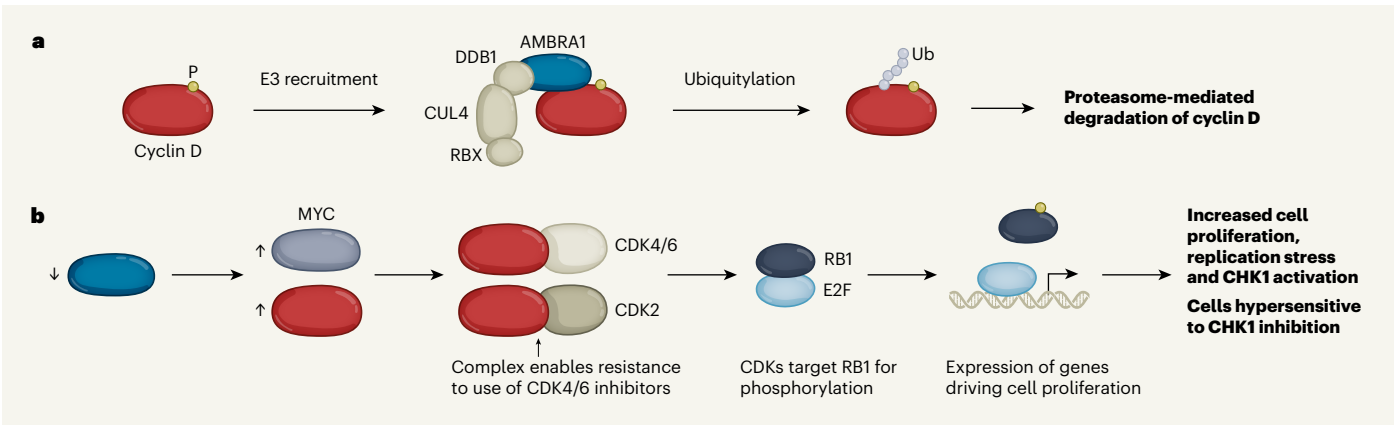

Figure 1 | The destruction of cyclin D protein. Simoneschi *et al.*[1], Chaikovsky *et al.*[2] and Maiani *et al.*[3] reveal how this key cell-cycle regulator is degraded in mammalian cells. **a**, Adding a phosphate group (P) targets cyclin D for degradation mediated by an E3 protein complex. This complex is called CRL4, and it includes the proteins cullin 4 (CUL4), RBX and DDB1. The protein AMBRA1 targets cyclin D to the E3. The E3 attaches chains of ubiquitin proteins (Ub) to cyclin D, marking it for degradation by a protein complex called the proteasome. **b**, AMBRA1 depletion leads to a rise in the levels of cyclin D and MYC proteins. Cyclin D binds to its usual partners, the enzymes CDK4 and CDK6, which phosphorylate the protein RB1. Phosphorylated RB1 releases E2F transcription factors to drive the expression of genes required for cell-cycle progression. In AMBRA1-depleted cells, cyclin D also forms complexes with the enzyme CDK2. These complexes enable cancer cells to resist treatment by CDK4/6 inhibitors. Cell proliferation driven by high cyclin D results in DNA damage, replication stress and activation of the enzyme CHK1. AMBRA1-depleted cells are hypersensitive to treatment with CHK1 inhibitors.

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Maiani and colleagues also show that AMBRA1 depletion elevates the levels of the transcription-factor protein N-MYC. This group previously showed[14] that AMBRA1 regulates the stability and activity of a related transcription factor, c-MYC. MYC-family proteins can upregulate the expression of D-type and E-type cyclin proteins[15], thereby accelerating cell-cycle progression.

These observations suggest that AMBRA1 might act as a tumour-suppressor protein. Indeed, mice with only one functional copy of the gene encoding AMBRA1 have a higher incidence of lung, liver and kidney tumours than do normal mice with two functional copies of the gene[14]. These new studies provide compelling evidence to support this idea.

The authors demonstrate that the *AMBRA1* gene is mutated in human cancers. As expected, given the ability of AMBRA1 to promote the degradation of cyclin D1, the authors report that the level of AMBRA1 in human tumours was inversely correlated with the level of cyclin D1. Moreover, low levels of AMBRA1 in tumours are associated with poor prognosis for people with cancer. The experimental inactivation of AMBRA1 in either human tumour cell lines or mouse cells engineered to have cancer-promoting mutations increased the tumour-forming potential of the cells, as assessed after injection into mice. Furthermore, the genetic ablation of *AMBRA1* boosted tumour formation in a mouse model of lung cancer driven by a mutant version of the *Kras* gene, and these AMBRA1-deficient tumours had a higher than normal level of D-cyclins. Collectively, these studies indicate that AMBRA1 normally restrains cell proliferation, largely by stopping D-cyclins from reaching high levels.

In addition, Chaikovsky, Simoneschi and their respective colleagues demonstrate that the loss of AMBRA1, and the concomitant increase in D-cyclins, results in decreased sensitivity of human tumour cells to CDK4/6 inhibitors. Intriguingly, these authors report that, in AMBRA1-depleted cells, rather than mainly partnering with CDK4/6, cyclin D1 also forms a catalytically active complex with the cyclin-dependent kinase enzyme CDK2, and that these complexes are insensitive to CDK4/6 inhibitors.

Maiani and colleagues also show that loss of AMBRA1, and the resulting rise of D-cyclins (and possibly also of other proteins, such as c-MYC), triggers DNA damage and replication stress, which is accompanied by the activation of a kinase enzyme called CHK1. Importantly, Maiani *et al.* report that AMBRA1-depleted cancer cells were hypersensitive to treatment with CHK1 inhibitors, which suggests a potential therapeutic opportunity to target AMBRA1-deficient tumours.

These exciting results raise several crucial questions. For example, does a decrease in AMBRA1 levels underlie existing and acquired resistance of human tumours to CDK4/6 inhibitors? And is the rise in D-cyclins that occurs with AMBRA1 depletion the sole factor responsible for the development of resistance to CDK4/6 inhibitors? Analyses of clinical trials in people with breast cancer[16–19] reveal no correlation between the presence of extra copies of the gene encoding cyclin D1, or the level of cyclin-D1 messenger RNA or protein in tumours, and a patient's response to CDK4/6 inhibitors. Indeed, Chaikovsky *et al.* found that human cancer cells driven to have higher than normal expression of D-cyclins did not fully recapitulate the characteristics of inhibitor resistance observed on AMBRA1 depletion. Perhaps other AMBRA1-regulated proteins, such as c-MYC, which can upregulate cyclin E protein and activate cyclin E–CDK2 complexes, contribute to treatment resistance.

"These observations suggest that AMBRA1 might act as a tumour-suppressor protein."

The observation by Chaikovsky *et al.* and Simoneschi *et al.* of the formation of CDK4/6-inhibitor-resistant cyclin D–CDK2 complexes in AMBRA1-depleted cells is intriguing. Such 'atypical' complexes were previously shown to underlie acquired resistance to CDK4/6 inhibition[20]. It is tempting to speculate that depletion of AMBRA1 somehow promotes the formation of these cyclin D–CDK2 complexes — and that this, along with an elevation of cyclin D levels, results in resistance to CDK4/6 inhibitors. A particularly exciting possibility, arising from Maiani and colleagues' work, is that CHK1 inhibitors can be used to treat CDK4/6-inhibitor-resistant tumours that have low levels of AMBRA1.

Further studies of the role of AMBRA1 in human cancer are necessary. Is the tumour-suppressive function of AMBRA1 mediated mainly by cyclin D1 or c-MYC, or is it also mediated by other targets? Tumour cells that no longer produce RB1 do not require D-cyclins for cell-cycle progression[4], so AMBRA1 loss would be expected to be observed in tumours that produce RB1 if D-cyclins are the main target of AMBRA1. It also remains to be determined whether AMBRA1 loss in human tumours is mutually exclusive with mutations affecting the C termini of D-cyclins, which would be expected to render D-cyclins resistant to AMBRA1-mediated degradation. Another unresolved issue is why, as Chaikovsky and colleagues report, low expression of AMBRA1 correlates with high levels of cyclin D1 and associates with poor survival of lung tumours that have specific types of genetic alteration, such as *Kras* mutations. This effect was not observed for lung tumours that instead have mutant versions of the gene encoding EGFR protein or have wild-type *Kras*.

Regardless of the answers to these questions, the impressive studies by Chaikovsky *et al.*, Maiani *et al.* and Simoneschi *et al.* improve our understanding of mechanisms governing cell-cycle progression.

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