inhibit the protein Rheb, the direct activator of mTORC1 (ref. 7). Growth-promoting signals inhibit TSC2, thereby blocking its inhibition of Rheb and activating mTORC1 (Fig. 1b). Conversely, growth-inhibitory signals promote the TSC2-mediated inhibition of Rheb and mTORC1. Individual signals influence TSC2 through distinct protein kinase enzymes, which phosphorylate specific serine or threonine amino-acid residues to modify the protein's function. Ranek and colleagues found that PKG1 phosphorylates serine residues at positions 1,364 and 1,365 in the human TSC2 protein (Fig. 1c). They further show that the phosphorylation of these two residues by PKG1 enhances the ability of TSC2 to inhibit Rheb and mTORC1, and is responsible for the sildenafil-induced inhibition of mTORC1 signalling seen in the hearts and isolated cardiomyocytes of mice.

Ranek and colleagues investigated how PKG1-mediated phosphorylation of TSC2 modulates the heart's response to pressure overload using two types of genetically modified mice. One type expressed a version of TSC2 in which the serine residue at position 1,365 had been changed to an alanine residue (dubbed the S1365A mutation), which cannot be phosphorylated. The other model expressed a version of TSC2 in which the serine residue at the same position had been changed to a glutamate residue (S1365E), a modification that mimics stable phosphorylation. Mice with the S1365A mutation had enhanced mTORC1 signalling in the heart and dramatically worsened cardiac hypertrophy and dysfunction in response to pressure overload, compared with wild-type mice. Furthermore, they were no longer protected by sildenafil treatment. By contrast, pressure overload did not activate the mTORC1 pathway in the hearts of mice with the S1365E mutation, which were protected from the harmful consequences of this stress.

Curiously, pressure overload had a dual effect. In addition to activating mTORC1 in the heart muscle and causing cardiac dysfunction, it triggered a modest increase in PKG1-mediated phosphorylation of TSC2, which attenuated mTORC1 activity and mitigated the effects of the stress. Increasing PKG1 activity using sildenafil further enhanced TSC2 phosphorylation and mTORC1 suppression, resulting in complete protection from cardiac hypertrophy induced by pressure overload (Fig. 1c).

Several points of clinical relevance arise from these findings. The yet unidentified mTORC1-activating signal that is triggered by pressure overload could be a potential therapeutic target. This signal probably leads to the phosphorylation of other amino-acid residues of TSC2, inhibiting the protein and consequently activating mTORC1 (Fig. 1b). A strong candidate is the peptide hormone endothelin-1, whose levels increase in humans with hypertension and which can induce cardiac hypertrophy in rodent models of the condition[8]. Endothelin-1 can stimulate mTORC1 activity in isolated cardiomyocytes[9], and Ranek *et al.* found that this effect could be blocked by PKG1-mediated phosphorylation of TSC2. Future work should also define the cellular processes downstream of mTORC1 that contribute to the development of cardiac hypertrophy. The findings of the current study suggest that chronic inhibition of autophagy and a failure to properly clear protein aggregates might be involved.

Given that chronic mTORC1 signalling is associated with a variety of disease states, the possibility that PKG1 activators would be useful in suppressing mTORC1 signalling in the heart or other affected tissues should be investigated. Could PKG1 activators, such as sildenafil, be more effective and safer than direct inhibitors of mTORC1, such as rapamycin and everolimus, in alleviating the harmful effects of uncontrolled mTORC1 signalling in specific tissues? The current study shows that TSC2 phosphorylation leading to mTORC1 inhibition is both necessary and sufficient for the protective effects of PKG1 activation in a setting of cardiac hypertrophy caused by pressure overload. However, other signals downstream of PKG1 are also likely to contribute to the protective effects of this pathway's activation[10]. This provides some rationale for the use of PKG1 activators, rather than mTOR inhibitors, for this condition. ■

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1. Ranek, M. J. *et al. Nature* **566**, 264–269 (2019).
2. Saxton, R. A. & Sabatini, D. M. *Cell* **168**, 960–976 (2017).
3. Ben-Sahra, I. & Manning, B. D. *Curr. Opin. Cell Biol.* **45**, 72–82 (2017).
4. Meikle, L. *et al. Hum. Mol. Genet.* **14**, 429–435 (2005).
5. Zhang, D. *et al. J. Clin. Invest.* **120**, 2805–2816 (2010).
6. Shende, P. *et al. Circulation* **123**, 1073–1082 (2011).
7. Dibble, C. C. & Manning, B. D. *Nature Cell Biol.* **15**, 555–564 (2013).
8. Miyauchi, T. & Sakai, S. *Peptides* **11**, 77–88 (2019).
9. Wang, L. & Proud, C. G. *Circ. Res.* **91**, 821–829 (2002).
10. Kim, G. E. & Kass, D. A. *Handb. Exp. Pharmacol.* **243**, 249–269 (2017).

This article was published online on 30 January 2019.

CANCER

One rogue agent suffices for genomic chaos

Genetic instability is a hallmark of cancer cells, and occurs when genes required for genomic maintenance are inactivated. It emerges that altering just one of the two copies of certain genes can drive genetic instability in yeast. SEE LETTER P.275

KATHERINE E. LARRIMORE & GIULIA RANCATI

Cancer is a disease of uncontrolled cell division that is fuelled by genetic instability — a state in which cells acquire mutations at an abnormally high rate. When normal cells are transforming into cancer cells, a common early event is the acquisition of mutations in a type of gene called a tumour-suppressor gene. If both of the two copies of a tumour-suppressor gene are inactivated in a cell, this decreases genomic stability and aids the acquisition of other cancer-initiating mutations. On page 275, Coelho *et al.*[1] report their studies in budding yeast (*Saccharomyces cerevisiae*), which indicate that, frequently, the disruption of just one copy of certain genes can be sufficient to trigger genetic instability.

The existence of tumour-suppressor genes was first proposed about 50 years ago to explain why, in some families, there is a puzzling pattern of inheritance of a type of cancer called retinoblastoma[2,3]. Clinical observations suggested that this cancer is caused by a type of mutation, known as a recessive mutation, in the gene *RB*. Such a mutation has an effect only if both copies of the gene are mutated in a cell. The 'two-hit' hypothesis was proposed[2] to explain the inheritance patterns of retinoblastoma. It suggested that if a cell inherits a recessive mutation in one copy of *RB*, it must also acquire a mutation in its other copy for cancer to develop. Subsequent research in mice[4] revealed that, although a two-hit scenario is common, the presence of a mutation in only one of the two copies of some tumour-suppressor genes (a condition termed haploinsufficiency) can suffice to trigger cancer formation.

Many other tumour-suppressor genes have been identified as being haploinsufficient in mice[5]. Moreover, a mutation in one copy of the *BRCA1* gene, which is associated with breast cancer, can cause genetic instability in the epithelial cells of human breast tissue grown *in vitro*[6], suggesting that haploinsufficiency of tumour-suppressor genes can kick-start cancer formation in human cells. But determining

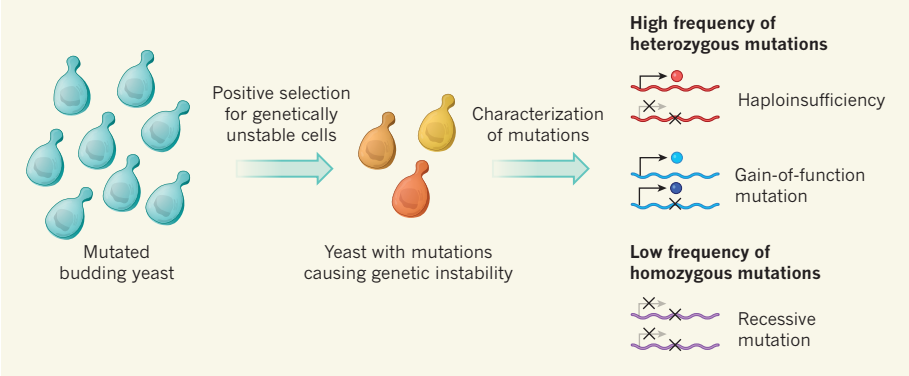

Figure 1 | Mutations that cause genetic instability. Coelho *et al.*[1] report the identification of mutations in budding yeast (*Saccharomyces cerevisiae*) that cause genomic abnormalities called genetic instability. The authors induced mutations in yeast cells, and used growth-limiting conditions to positively select for surviving cells. Such cells might have acquired their capacity for survival in these conditions as a result of changes arising from genetic instability. Characterization of the mutations responsible for generating genetic instability revealed a high frequency of mutations in one of the two copies of a particular gene, which is termed a heterozygous mutation. Such a mutation can affect a cell if it causes a decrease in production of the protein encoded by the gene, a situation known as haploinsufficiency, or if it results in a version of the encoded protein that functions in an unusual way, called a gain-of-function mutation. Coelho and colleagues found that, of the mutations they identified as being responsible for genetic instability, homozygous mutations (which affect both copies of a particular gene) were less frequent than were heterozygous mutations. The type of homozygous mutations that have an effect only when both copies of a gene are mutated are termed recessive mutations, and some of these were found by the authors. This insight into the relative frequency of heterozygous and homozygous mutations associated with genetic instability in yeast might have relevance for understanding the mechanisms by which cancer cells acquire genetic instability.

how commonly genetic instability arises from inactivation of either one or both versions of a gene is challenging, and cannot be easily assessed using current mouse models of cancer or by analysing DNA sequences of tumours obtained from people who have cancer. This is because, if cell samples are obtained after the cancer has arisen, and both copies of a gene have mutations, it is difficult to know whether one or both of the mutations occurred before the cancer developed.

To address this, Coelho and colleagues devised a way of identifying mutations that cause genetic instability in yeast. They used a system in which there is an evolutionary selective pressure for the development of cells that can generate genetic changes enabling them to survive treatment with drugs that usually limit growth. A key advantage of this system is the ease with which characteristics that have a predictable, Mendelian pattern of genetic inheritance can be followed. This makes it easy to determine whether mutations occurred in one or both copies of a gene — states respectively known as heterozygosity and homozygosity. The authors found that most of the yeast cells in which genetic instability had occurred had inherited this capacity through a mutation in only one copy of the gene responsible (Fig. 1). Whole-genome sequencing of cells confirmed this and also revealed the identity of the mutations.

The authors individually introduced DNA sequences corresponding to 16 of these mutations into wild-type yeast cells, and found that this was sufficient to cause genetic instability. Further testing revealed that ten of these were loss-of-function mutations in which the encoded protein has reduced function, doesn't function at all or isn't made, whereas the other six had a profile consistent with gain-of-function mutations, in which the protein encoded by the mutant gene functions in an abnormal way. Of the genes identified that caused genetic instability when mutated, 57 have related versions in humans, and 10 of these have previously been linked to cancer. The other 47 were mainly genes that encode proteins involved in processes such as protein quality control and cellular transport mechanisms. These genes have not previously been connected to the generation of genetic instability.

The authors tested the effect of inactivating the human versions of six of these genes *in vitro* in haploid cancer cells — cancer cells that contain only one copy of each gene. They found that the mutations caused an increase in genetic instability, confirming the power of the approach and suggesting that such mutations might underlie cancer development. A follow-up study could test diploid versions of the cells — those that have two copies of each gene — to confirm the role of heterozygous mutations in driving genetic instability.

Another future direction for research might be to investigate the mechanisms that link genetic instability to mutations in genes involved in protein quality-control processes. One possibility is that mutations in such genes cause a decrease in the degradation of key enzymes that affect DNA synthesis, modification or repair, or that affect proteins important for cell division. If the pattern of degradation changes for such enzymes, the resulting interference with protein turnover might have negative consequences for processes in which the enzymes function. For example, mutations in genes that encode components of a protein complex called the proteasome, which has a key role in protein degradation, cause genetic instability in yeast by affecting the turnover of an enzyme that repairs breaks in DNA[7]. Another possibility is that genes involved in quality-control processes have a more direct role than previously appreciated in regulating DNA metabolism or cell division.

It is also interesting to consider whether, during cancer development, heterozygous mutations conferring genetic instability might subsequently be lost as the cancer evolves. Although instability might fuel evolutionary processes and increase tumour-cell fitness under adverse circumstances, it could also cause mutations that decrease the overall fitness of the cancer-cell population in the absence of stress. Depending on the evolutionary selective pressure faced by cancer cells, the gain or loss of genetic instability might be selected for according to circumstances, either to increase the variation in the cancer-cell population when the cells are facing an evolutionary selective pressure, or to ensure that useful variants are retained.

One way of quantifying such changes would be to take samples of cancer cells from a tumour at different time points, and to track genomic evolution during the acquisition of resistance to anticancer drug treatment. Heterozygous mutations conferring genetic instability would be predicted to be enriched at earlier stages of an evolutionary selection process, but there might be a reversion to a homozygous wild-type condition once other mutations have arisen that provide the adaptive features the tumour needs. Elucidating such dynamics will be a challenging task, but the outcome should be rewarding because it will increase our understanding of the competition that occurs between different cancer-cell lineages as a tumour develops. ■

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1. Coelho, M. C., Pinto, R. M. & Murray, A. W. *Nature* **566**, 275–278 (2019).
2. Knudson, A. G. Jr *Proc. Natl Acad. Sci. USA* **68**, 820–823 (1971).
3. Nordling, C. O. *Br. J. Cancer* **7**, 68–72 (1953).
4. Fero, M. L., Randel, E., Gurley, K. E., Roberts, J. M. & Kemp, C. J. *Nature* **396**, 177–180 (1998).
5. Inoue, K. & Fry, E. A. *Adv. Med. Biol.* **118**, 83–122 (2017).
6. Konishi, H. *et al. Proc. Natl Acad. Sci. USA* **108**, 17773–17778 (2011).
7. Ben-Aroya, S. *et al. PLoS Genet.* **6**, e1000852 (2010).

This article was published online on 30 January 2019.