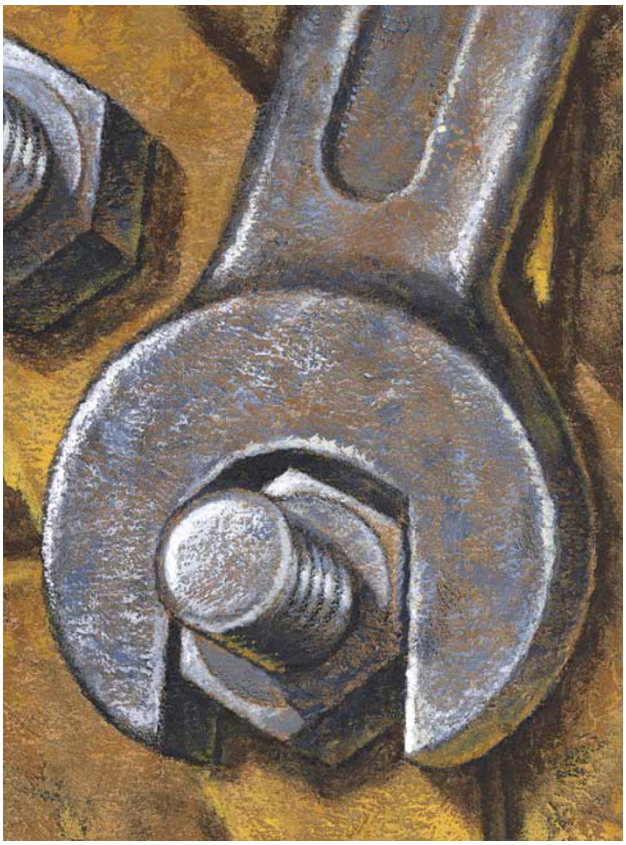
CARDIOVASCULAR DISEASE

Plumbing for the heart



Fixing a leaky calcium channel could be a promising approach for protecting against life-threatening cardiac arrhythmias, according to a recent paper in *Science*. The research by Marks and colleagues shows that an experimental drug known as JTV519 enhances the binding of the protein calstabin-2 to a calcium channel present in heart cells, thereby stabilizing the channel in its closed state and preventing potentially dangerous calcium leakage.

Calstabin-2 forms part of the ryanodine receptor–calcium-release channel (RyR2) complex that regulates calcium release from the sarcoplasmic reticulum (SR). Calstabin-2, which is bound to RyR2, helps maintain the closed state of the channel during the diastolic (resting) phase of the cardiac cycle. But in patients with heart failure, and in some inherited forms of exercise-induced sudden cardiac death (SCD), depletion of calstabin-2 from the RyR2 complex has been shown to cause calcium leakage that can trigger fatal ventricular tachyarrhythmia (VT).

Successful prevention or treatment of SCD has been hindered by an incomplete understanding of the molecular mechanisms that cause VT. However, JTV519 was recently shown to reduce diastolic SR calcium leakage in an animal model of heart failure, and now Marks and colleagues have clarified the underlying mechanism in a series of experiments using mice that are either partially or completely deficient in calstabin-2.

When calstabin-$2^{+/-}$ mice were subjected to an exercise protocol designed to induce stress, 100% developed VT and 89% subsequently died. By contrast, no arrhythmias were observed in calstabin-$2^{+/-}$ mice that were treated with JTV519 before exercise. However, in calstabin-2-null mice, the protective effect of JTV519 was negated: all treated and untreated null mice in the study developed VT and died. This indicates that JTV519 exerts its protective effect via calstabin-2, but to characterize this further the authors turned their attention to the relationship between exercise, phosphorylation of RyR2 and calstabin-2 depletion.

Exercise is known to induce protein kinase A (PKA) phosphorylation of RyR2, which in turn triggers the dissociation of calstabin-2 from RyR2. Correspondingly, the value of P_O (probability that channels will be open) for RyR2 significantly increases in calstabin-$2^{+/-}$ mice during exercise, compared with calstabin-$2^{+/+}$ mice, when the channels are examined under conditions that

WHOLE ORGANISM SCREENING

Zebrafish reduce drug discovery gridlock

One of the problems with approaches to conventional drug discovery is the need for information on an appropriate molecular target, when more often than not the pathways that need to be targeted for a therapeutic outcome are not obvious. In the May issue of *Nature Biotechnology*, Peterson *et al.* use a phenotype-based screen of a zebrafish model of aortic deformities to identify pathways and potential drug leads that correct the disease.

The zebrafish mutation gridlock (*grl*), which affects the gene *hey-2*, disrupts aortic blood flow in a similar way to aortic coarctation in humans, a common congenital cardiovascular malformation. The authors screened for chemical suppressors of *grl* by arraying mutant embryos in 96-well plates and exposing them to small molecules from a structurally diverse chemical library. After 48 hours of treatment, two small molecules out of 5,000 tested suppressed the gridlock phenotype, restoring normal blood circulation. The molecules, 4-[2-(4-methoxy-phenylsulphanyl) ethyl] pyridine and 4-[2-(4-nitro-phenylsulphanyl) ethyl] pyridine, were structurally related compounds. The former (also called GS4012) was more potent and was used for all subsequent experiments.

To elucidate the biological mechanism, the authors treated wild-type embryos with GS4012 and measured the expression level of genes known to regulate vasculogenesis and aortic development. Significant and dose-dependent increases in mRNA levels of vascular endothelial growth factor (VEGF) were observed in response to drug treatment. The ability of GS4012 to cause overexpression of VEGF could be the mechanism that rescues the mutant, as *grl* is thought to act downstream of VEGF signalling. When tested on primary human endothelial cells, the drug caused an increase in tubule formation, compared with controls.

This work demonstrates that phenotype-based chemical screens in vertebrate models can be valuable in identifying drug leads; however, whether the leads will be developed in light of the scant knowledge of the specific target remains to be seen. Drugs have been developed in the absence of a clear target, such as ezetimibe (Zetia; Merck) and metformin (Glucophage; Bristol-Myers Squibb), but in general it is likely that drug leads with known targets will receive higher priority than those without.

Melanie Brazil

References and links

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FURTHER READING Patton, E. E. & Zon, L. I. The art and design of genetic screens: zebrafish. *Nature Rev. Genet.* **2**, 956–966 (2001) | MacRae, C. A. & Peterson, R. T. Zebrafish-based small molecule discovery. *Chem. Biol.* **10**, 901–908 (2003)

simulate diastole. The authors showed that JTV519 prevents the depletion of calstabin-2 that is observed in mice during exercise and significantly reduced P_O in calstabin-$2^{+/-}$ mice but not in calstabin-2-null mice. However, JTV519 had no direct effect on this phosphorylation, indicating that it increases the affinity of calstabin-2 for RyR2 even when the channel is phosphorylated by PKA. Indeed, further experiments showed that the affinity of calstabin-2 for both wild-type and a mutant, constitutively PKA-phosphorylated RyR2 was increased in the presence of JTV519, regardless of phosphorylation status of the channel.

The authors speculate that calcium leakage resulting from calstabin-2-deficient RyR2 alone might not be sufficient to cause SCD, but that when combined with exercise, the resulting phosphorylation of RyR2 and subsequent dissociation of already depleted levels of calstabin-2 is enough to cause a fatal arrhythmia. JTV519 has therefore provided both an insight into this mechanism and a potential therapeutic strategy to prevent SCD.

Joanna Owens

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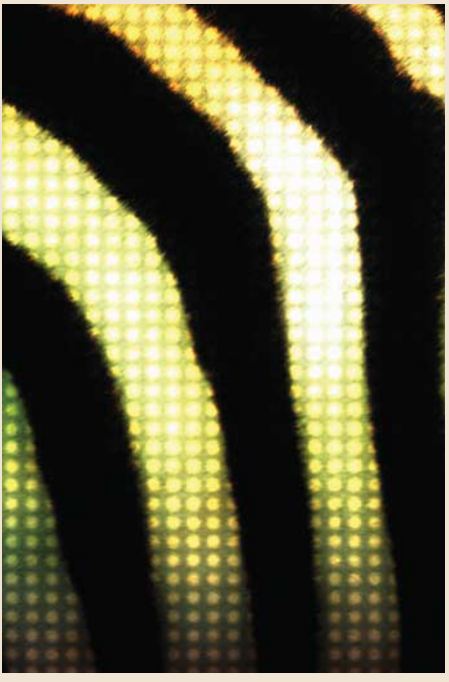

CHEMICAL GENETICS

New use for the cellular dustbin

Protein degradation is essential in cellular homeostasis, and the proteasome — a large protease complex in the cell that destroys appropriately tagged proteins — has a key role in this process. Now, as reported in the *Journal of the American Chemical Society*, Craig Crews and colleagues have harnessed the proteasome by using small molecules to direct the selective degradation of particular proteins in whole cells, thereby providing a novel general method to study the effects of the removal of selected proteins.

Proteins are normally tagged for destruction by the proteasome with a polymer chain formed from a small protein called ubiquitin, which is attached in a three-part process. First, the carboxyl terminus of ubiquitin is activated by an enzyme known as E1. The activated ubiquitin is then transferred to a ubiquitin-conjugating enzyme (E2). Finally, ubiquitin is transferred to a lysine of the protein substrate that is specifically bound by a ubiquitin ligase (E3). Successive attachment of ubiquitin to internal lysines in previously added ubiquitins results in the formation of the polyubiquitin destruction tags.

Crews and colleagues have exploited this process by developing bifunctional molecules — dubbed PROTACs — that comprise a ligand for the target protein, a linker and a ligand for an E3 ligase. By bridging the target protein and an E3 ligase, the PROTAC molecule initiates the ubiquitination of the target protein, leading to its proteasome-mediated degradation.

The authors had previously provided proof of principle that this strategy worked *in vitro* on one stable protein, and so set out to investigate whether it would be capable of significantly reducing the level of proteins in whole cells simply by adding PROTACs to the cells. They designed a PROTAC molecule that consisted of a heptapeptide that binds to an E3 ligase, a linker, a known ligand for a mutant form of FK506-binding protein (FKBP12) and a poly-D-arginine tag, as such tags are known to facilitate translocation of peptides into cells.

To assess the impact of this molecule on protein levels after addition to cells, they monitored the fluorescence from a fusion protein of FKBP12 and enhanced green fluorescent protein. Fluorescence was lost in cells treated with the PROTAC, but not with either of the two protein ligands separately, confirming that the PROTAC worked as expected. Further analogous experiments — this time targeting the androgen receptor by using a second PROTAC incorporating dihydrotestosterone as the protein ligand — confirmed the robustness of the approach for inducing intracellular protein degradation.

The strategy described should in principle be applicable to 'knocking out' any protein for which an appropriate ligand is available, potentially providing a convenient and rapid complementary approach to conventional genetic knockouts for studying protein function. Indeed, libraries of PROTACs might prove useful in chemical-genetic screens for phenotypic effects. This approach might also allow more temporal or dosing control than gene inactivation at the RNA level with RNA interference (RNAi), a feature that could be important if molecules based on the PROTAC principle were developed as potential therapeutics for diseases that depend on the overexpression or presence of a particular protein.

Peter Kirkpatrick

References and links

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