To confirm a role for TDP-43 in muscle formation, the authors generated mice whose muscle stem cells lacked one of two copies of the gene that encodes the protein. Lowering the level of TDP-43 in this way led to a decrease in the diameter of the muscle fibres generated in response to injury, indicating that TDP-43 is important for full muscle regeneration — probably because it somehow regulates the expression of muscle mRNAs. However, this experiment does not prove that myo-granule formation is necessary for TDP-43 function in muscle regeneration; reducing TDP-43 levels causes cellular dysfunction in many cell types, but Vogler *et al.* report myo-granules only in myotubes.

Regardless of the physiological function of TDP-43 myo-granules, the authors' data beg the question of whether these structures can eventually turn into disease aggregates. To investigate this possibility, the group turned to mice carrying a mutated form of the gene *VCP* that can cause ALS, FTD and IBM in humans[7]. The mutant mice, in which muscle, brain and bone tissue degenerates[5], had many more myotubes harbouring TDP-43 myo-granules than did wild-type mice. This suggests that *VCP* mutations might increase the risk of tissue degeneration by increasing the prevalence of myo-granules. In this scenario, perhaps small seeds of TDP-43 from myo-granules could be transported to the nerves that innervate muscle, where they might initiate a cascade of TDP-43 aggregation. Indeed, the earliest signs of neurodegeneration in ALS seem to originate at the nerve terminals adjacent to muscle, resulting in a 'dying-back' phenomenon that eventually reaches the main body of the neuron, which houses the nucleus[8].

The differences between TDP-43 disease aggregates and myo-granules are as interesting as the similarities. Unlike myo-granules, most TDP-43 disease aggregates seem to have an amorphous structure, although some do have amyloid-like characteristics[9]. Moreover, the disease aggregates seem to be irreversible, whereas myo-granules disassemble as muscle cells mature. Because of this, myo-granules could provide an opportunity to investigate how strongly bound aggregate structures are disassembled. Factors that promote the disassembly of myo-granules might also be effective at clearing disease-associated aggregates.

Vogler and colleagues' findings raise an intriguing question. Strenuous exercise and weight training stimulate repeated rounds of muscle growth and repair — could this activity increase the production of TDP-43 myo-granules, increasing the propensity of TDP-43 to aggregate and so leading to diseases such as ALS? Indeed, there is some evidence for increased prevalence of ALS in elite athletes[10,11]. However, much more evidence for the role of myo-granules and more human data will be needed before such a link can be assumed.

This paper sets the stage for future work characterizing the physiological function and regulation of TDP-43 myo-granules, and for investigating how these complexes might contribute to disease. There are other examples of amyloid-like protein complexes that form in healthy cells[12,13], but Vogler *et al.* describe the first that are made up of a protein that can also aggregate in disease. The race is on to search for more of these kinds of functional granule in other cell types. The idea that amyloid-like structures might have beneficial roles, rather than simply being associated with disease, represents a change in our understanding of these protein aggregates. Myo-granules provide a unique opportunity to unravel the differences between a safe and a dangerous aggregate. ■

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THERAPEUTIC RESISTANCE

A new road to cancer-drug resistance

The discovery of a mechanism that leads to cancer-therapy resistance highlights the many ways that tumour cells can adapt to survive — and reveals the limitations of categorizing patients by their gene mutations. SEE ARTICLE P.522

KATHARINA SCHLACHER

The development of resistance to cancer therapy is a major predictor of patient mortality. Therefore, understanding resistance mechanisms is key to improving therapeutic outcomes. On page 522, He *et al.*[1] report their discovery of a resistance mechanism in ovarian-cancer cells that contain a mutant version of the *BRCA1* gene.

Mutations in *BRCA1* and *BRCA2* genes can cause breast and ovarian cancer by inactivating either of two major biological pathways that ensure genome stability. One of the pathways repairs DNA double-strand breaks through a process called homologous recombination[2] (HR). The other process is called fork protection[3,4], and safeguards newly synthesized DNA at structures called stalled forks that arise during DNA replication.

In HR repair, an essential bottleneck step is the processing (resection) of double-strand breaks by nuclease enzymes to produce single-stranded (ss) DNA. BRCA1 acts as a key regulator protein that coordinates the recruitment of the nucleases, which include the MRE11–RAD50–NBS1 protein complex. BRCA1 also has a second role in HR repair: it recruits BRCA2, which in turn loads the RAD51 protein onto the ssDNA. RAD51 then assists in the binding of the ssDNA to a complementary strand that serves as a template for error-free repair.

Cancer cells that have certain *BRCA1* or *BRCA2* mutations cannot repair double-strand breaks caused by anticancer drugs currently used in the clinic, and so die when treated. Such drugs include cisplatin and PARP inhibitors (PARPi, drugs that specifically target BRCA-mutant tumours by taking advantage of their break-repair defects[5]). However, cancer cells can acquire strategies to circumvent the drugs' actions, causing resistance and limiting the use of these initially effective drugs.

In their rigorous study, He *et al.* used a gene-editing screening method[6] to identify resistance mechanisms in *BRCA1*-mutant ovarian-cancer cells. A known resistance pathway in both *BRCA1*-mutant and *BRCA2*-mutant cells is the restoration of BRCA function by re-mutating the original *BRCA* mutation (see ref. 7, for example; Fig. 1a). A second mechanism is drug avoidance, in which a membrane protein pumps the drug out of the cell or reduces its uptake[8]. He and colleagues' screen correctly identified a membrane protein implicated in the uptake of cisplatin by tumour cells as a contributor to resistance, verifying

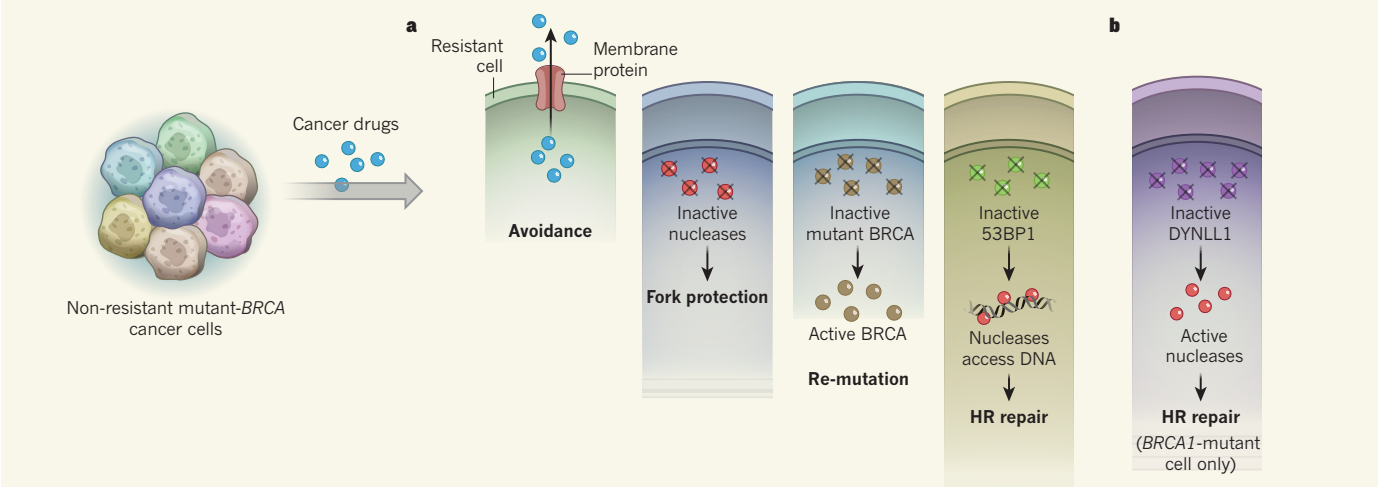

Figure 1 | Mechanisms of drug resistance in cancer cells that contain *BRCA* mutations. Many cancer cells have mutations in the *BRCA1* or *BRCA2* genes. These mutations inactivate a DNA-repair pathway that involves a process called homologous recombination (HR), or a process known as fork protection that is involved in DNA replication. **a**, *BRCA*-mutated cancer cells have developed many different paths to resist being killed by cancer drugs, including: drug avoidance by pumping drugs out of the cell through a membrane protein; restoration of fork protection by inactivating nuclease enzymes; re-mutating the original *BRCA* mutation to restore the functions of the BRCA proteins; and restoration of HR repair, for example by inactivating the 53BP1 protein in *BRCA1*-mutant cells to allow nucleases to access DNA. Here, the resistant cells are derived from non-resistant cells of the same colour. **b**, He *et al.*[1] report that in *BRCA1*-mutant cells, but not in *BRCA2*-mutant cancer cells, inactivation of the DYNLL1 protein activates nucleases and thus restores HR repair.

the suitability of the authors' approach.

Importantly, one of the top gene 'hits' identified by the screen as causing resistance to both cisplatin and PARPi was *DYNLL1*. The DYNLL1 protein acts in many cellular processes[9], including intracellular transport and motility, and also inhibits the enzyme nitric oxide synthase (which produces the cell-signalling molecule nitric oxide), but had not been previously implicated in cancer-drug resistance. Deciphering how its inactivation leads to resistance therefore seemed a daunting task.

The authors robustly established that DYNLL1 acts as a negative protein regulator of DNA-end-processing nucleases — it directly interacts with MRE11 and thereby keeps its nuclease activity in check. Inactivation of DYNLL1, therefore, unleashes the nuclease activity of MRE11, even when BRCA1 is not there to help guide it to breaks, and so restores the first of the two HR-repair functions normally carried out by BRCA1 (Fig. 1b).

Conceptually, drug resistance associated with DYNLL1 inactivation is analogous to that caused by inactivation of 53BP1 — another protein that inhibits DNA-end resection, in this case by blocking the access of nucleases to DNA. Inactivation of 53BP1 has been reported to restore resection and therefore resistance in *BRCA1*-mutant cells, but not in *BRCA2*-mutant cells[10,11]. Moreover, DYNLL1 is known[12] to interact with 53BP1. Yet, unexpectedly, He *et al.* show that resistance associated with DYNLL1 inactivation does not occur through loss of the 53BP1–DYNLL1 interaction.

He and colleagues' study highlights the intricacy of distinct gene functions in cancer-drug resistance, and the importance of defining biological mechanisms and activities to predict whether tumour cells will be killed. In this case, DYNLL1 inactivation reactivates resection, which is ablated in *BRCA1*-mutant cells, but not in *BRCA2*-mutant tumour cells. DYNLL1 inactivation, therefore, results in resistance in *BRCA1*-mutant cells, but not in *BRCA2*-mutant cells.

Another resistance mechanism that separates BRCA1 and BRCA2 functions has been reported in *BRCA2*-mutant ovarian-cancer cells[13], where inhibition of the EZH2 enzyme reduces the recruitment of the MUS81 nuclease to replication forks. Notably, this does not restore HR repair, but instead restores fork protection and the survival of *BRCA2*-mutant cells, and not of *BRCA1*-mutant cells. By contrast, EZH2 inhibition increases the sensitivity of *BRCA1*-mutant breast cancers to PARPi (ref. 14). Yet restoration of fork protection by inhibition of MRE11 results in resistance to PARPi and cisplatin in both *BRCA1*- and *BRCA2*-mutant cells, even when HR repair remains defective[15]. Thus, it is tempting to suggest that tumour-cell heterogeneity[16] — the existence of tumour-cell subtypes that stem from the same mutation, but which have mutated further to form distinct subpopulations — could partly arise as a result of cells making individual 'decisions' about which survival mechanisms to use, including HR repair, fork protection or both.

Although BRCA1 promotes resection during HR repair, it can also prevent the degradation of newly synthesized DNA by nucleases during fork protection, through an unknown mechanism. These dual, and seemingly opposing, modes of action raise the possibility that restoration of HR repair could promote tumour-cell survival even when fork protection is dysfunctional. Similarly, DYNLL1 inactivation might in fact cause defects in fork protection by promoting excessive MRE11 activity at replication forks, irrespective of *BRCA* mutations. Future studies will be crucial to dissect the apparently opposing nuclease processes at breaks and forks, and their effects on tumour-cell survival, as a possible nexus point for therapeutic intervention.

The emergence of diverse mechanisms for cancer-drug resistance demonstrates that cancer cells respond distinctively to individual defects of molecular function — rather than to an overall genetic defect — to rebalance the cellular homeostasis that ensures their survival. He *et al.* identified DYNLL1 inactivation as a resistance mechanism to cisplatin and PARPi; although both drugs cause double-strand breaks, their mode of action differs. In addition, other commonly used anticancer drugs, including gemcitabine, 5-fluorouracil and hydroxyurea, mainly disrupt replication reactions. Researchers, therefore, should expect to identify many different ways in which resistance can develop. These roads to resistance might require the restoration of replication processes, repair processes or both. The sequential use of different cancer therapies when an initial treatment is not successful is routine practice, but could lead to the development of multiple resistance mechanisms, and ultimately to resistance to any of the therapies.

People with cancer who have *BRCA1* and *BRCA2* mutations are currently grouped together in many genome studies and when considering treatment options, despite increased understanding of the molecular and genetic distinctions. He and co-workers' study suggests that molecular function, rather than genotype function — in this case, the specific

role of BRCA1 in resection, rather than its overall role in HR repair or in fork protection — dictates the cellular outcomes. More broadly, these results suggest that personalized-medicine strategies should be considered that take into account molecular functions in individuals, rather than categorizing people solely by genotype. ■

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OPTOELECTRONICS

Efficiency breakthrough for radical LEDs

A strategy for using organic free radicals to make light-emitting diodes circumvents the constraints that limit the efficiency with which other organic LEDs convert electric current into light. SEE LETTER P.536

TETSURO KUSAMOTO & HIROSHI NISHIHARA

Light-emitting devices made from organic materials have the potential to be thin, flexible and lightweight, and might therefore be used in a variety of applications — including foldable display screens, 'smart' wallpaper incorporating digital devices, and windows that could be converted into illuminated panels at the flick of a switch. On page 536, Ai *et al.*[1] report the development of organic light-emitting diodes (OLEDs) that use free radicals as the emitter and convert electrons into light with high efficiency. The efficiencies of other types of OLED are generally limited by quantum-mechanical effects, but radical-based OLEDs (ROLEDs) don't have this constraint, owing to the electronic state of the radicals. The authors' ROLEDs have the highest emission efficiency obtained so far among LEDs that emit light in the deep-red and infrared regions of the electromagnetic spectrum.

Several types of LED are being actively developed because they are expected to produce displays that have higher brightness, colour purity, contrast and resolution than conventional lighting devices, while using less energy. OLEDs, in particular, have become familiar in the past decade, because they are used in the displays of mobile phones and televisions. Such displays perform better in several respects (such as contrast and colour reproducibility) than do liquid-crystal displays, which are currently used in many electronic devices.

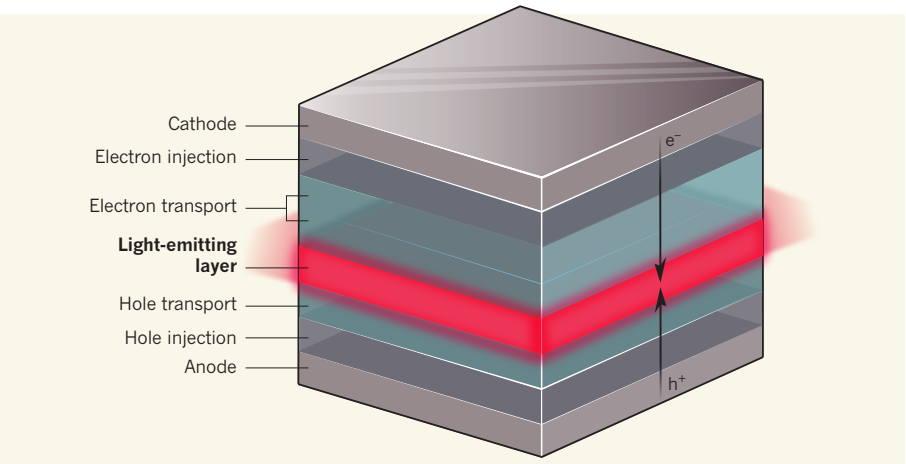

Figure 1 | An efficient radical-based organic light-emitting diode (ROLED). Ai *et al.*[1] report two organic free radicals that can be used in multilayered light-emitting diodes. Electrons (e^-) and holes (h^+; quasiparticles formed by the absence of electrons in an atomic lattice), which are produced by a cathode and an anode, respectively, pass through injection layers and transport layers before merging (recombining) on radical molecules in the light-emitting layer. This recombination produces light in the deep-red and infrared regions of the electromagnetic spectrum. Photons are produced from electrons in the light-emitting layer with almost 100% efficiency. The maximum external quantum efficiency of the device (the ratio of the number of photons that leave the LED to the number of electrons injected into it) is 27%, the highest such efficiency of any LED that emits deep-red and infrared light.

OLEDs were first reported[2] in 1987, and typically have a multilayered structure: a layer of material that contains light-emitting molecules is sandwiched between layers that transport electrons and holes (positively charged quasiparticles formed by the absence of electrons in atomic lattices), which, in turn, are sandwiched by electrodes as the outermost layers (Fig. 1). Additional layers that enable efficient injection of holes and electrons from the electrodes into the transport layers are also sometimes used. When an electric field is applied between the two electrodes, holes and electrons are injected and merge (recombine) on emitter molecules in the light-emitting layer to generate photons. The structure of the emitter molecule determines the colour of the emission.

One problem that still needs to be overcome for OLEDs is their low efficiency, which is quantified by the external quantum efficiency (EQE) — the ratio of the number of photons that leave the device to the number of electrons injected into it on the application of an electric field. The EQE is, in turn, proportional to two factors: the internal quantum efficiency (IQE), which is the efficiency with which photons are generated in the light-emitting layer from injected electrons; and the light outcoupling efficiency, which is the ratio of the number of photons that exit the device to the number generated within it. The value of the outcoupling efficiency is typically 20–30% (ref. 3). Quantum mechanics dictates that the IQE of conventional OLEDs based on fluorescent molecules is limited to 25% (ref. 4). The remaining 75% of efficiency is lost through recombination pathways that don't result in light emission. The EQEs of such OLEDs are therefore 5–6% at best.

Several groundbreaking methods have been established to solve the efficiency problem. For example, the IQE of OLEDs has been increased to nearly 100% by using phosphorescence (rather than fluorescence) as the light-emitting process[5], or by using a heat-activated