families as new metagenomes were added to their analysis. Instead, the detection of protein families increased exponentially, warranting an array of follow-on studies.

The distribution of protein families across Earth's categories of ecosystem (biomes) presented by Pavlopoulos and colleagues corroborates the findings of previous investigations regarding the distribution of microbial genes[8]. Some biological entities, however, were particularly rich sources of newly discovered protein families, including viruses, as Pavlopoulos *et al.* report, and microbes called Asgardarchaeota, as presented by Rodríguez del Río and colleagues. The latter are a group of microorganisms called archaea that are closely related to the first ancestor of eukaryotes. As such, studying their proteins might reveal new insights into the evolution of the eukaryotic cell[9].

One major challenge in exploring the wealth of previously unknown protein families encoded in genomes of natural samples is the identification of eukaryotic genes in metagenomes. Although certain algorithms exist for the recovery of eukaryotic genomes from metagenomes, accurately predicting eukaryotic genes in mixed DNA sequences — equivalent to Pavlopoulos and colleagues' method of identifying microbial genes — is still not possible bioinformatically. Once this shortcoming is overcome with the development of new algorithms, scientists will substantially expand the protein 'sequence space' and will identify protein families of unknown function that drive the ecology and evolution of eukaryotes.

The greatest advance in painstakingly organizing the protein families of nearly 27,000 metagenomes and across the tree of life lies in the identification of ecosystem-specific protein clusters that differ in terms of their presence or absence, or relative abundance between varying conditions of a given ecosystem — for example, between the contexts of health or disease. Applying this strategy to examine microbial data for healthy people and those with colorectal cancer, Rodríguez del Río and colleagues found that specific unknown protein families were enriched in the gut bacteria of people with cancer. These protein families were associated with microbial motility, adhesion and invasion potentially of human tissue, as revealed through genomic-context analysis. Harnessing this approach in other fields of research should be extremely helpful for deciphering the different functions of sample sets, in the hope of identifying new targets for biochemical analyses to shed light on a tiny fraction of the microbial dark matter.

Identifying differences in microbial communities (microbiomes) that might explain, for example, the disease state of a person, rely heavily on comparing which species are present and how abundant they are (the taxonomic composition), and examining genes that are associated with certain functions. Finding specific but differentially abundant protein families of unknown function, as demonstrated by Rodríguez del Río and co-workers, has the potential not only to replace current marker-gene-based approaches for differentiating microbiomes but also to advance microbiome research to a new and causality-driven level.

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Cancer

Natural inhibitor found for cell death by ferroptosis

Donna D. Zhang

The discovery that an evolutionarily conserved molecule used to make cholesterol also acts as a defence against a cell-death mechanism called ferroptosis might lead to new ways to treat cancer and other clinical conditions. **See p.401 & p.411**

Biology remains nothing short of astonishing, as researchers unveil the underpinnings of its myriad systems, especially those that are involved in protecting against cell death. On pages 401 and 411, respectively, Freitas *et al.*[1] and Li *et al.*[2] shed light on a regulated form of cell death called ferroptosis, which is driven by an iron-dependent modification of lipids in cellular membranes. The results bring into sharp focus an unexpected hero, the molecule 7-dehydrocholesterol (7-DHC).

The term ferroptosis was coined in 2012 (ref. 3). This cell death encompasses a variety of processes that include lipid oxidation by the action of reactive molecules called radicals (versions of molecules that have an unpaired electron) and the fragmentation of lipids at cellular membranes, culminating in membrane disruption, shrunken mitochondrial organelles and swelling of cells ('ballooning'). Ferroptosis occurs when there are problems in the regulation of normal iron levels (iron homeostasis) and in the oxidation of lipids. Preventing ferroptosis might be beneficial in alleviating neurodegenerative and kidney diseases, and activation of ferroptosis can kill cancer cells[4–7].

Freitas *et al.* and Li *et al.* report that 7-DHC, a molecule in the cholesterol-synthesis pathway (Fig. 1), acts to suppress ferroptosis. Both teams independently discovered the anti-ferroptotic role of the cholesterol-synthesis pathway. The authors reveal that several enzymes in this pathway function as potential suppressors of ferroptosis. However, one of the enzymes, DHCR7, which catalyses the reaction that converts 7-DHC to cholesterol, was found to promote ferroptosis. This indicates that 7-DHC, produced by the enzyme SC5D and used by DHCR7, operates as a key protection against ferroptosis.

Both teams then explored the mechanism of action of 7-DHC in more detail. They highlighted a key characteristic of its structure — a part that is described as a conjugated double bond in the sterol B-ring. This component of its structure enables 7-DHC to absorb radicals, thereby reducing lipid fragmentation driven by an oxidation process called peroxidation.

Both teams recognized that it is mainly lipid components called phospholipids — especially if these are fragmented into smaller pieces — that can initiate ferroptosis. These findings underscore the protective function of 7-DHC, especially in scenarios in which ferroptosis might otherwise occur if this molecule wasn't present.

Most remarkably, the two teams also found that the molecule ergosterol, which is found in yeast and fungi and has structural similarity to 7-DHC, also offers protection against ferroptosis. This finding suggests that the anti-ferroptotic effect of a molecule in the cholesterol-synthesis pathway might be evolutionarily conserved, serving to safeguard a variety of organisms from ferroptosis.

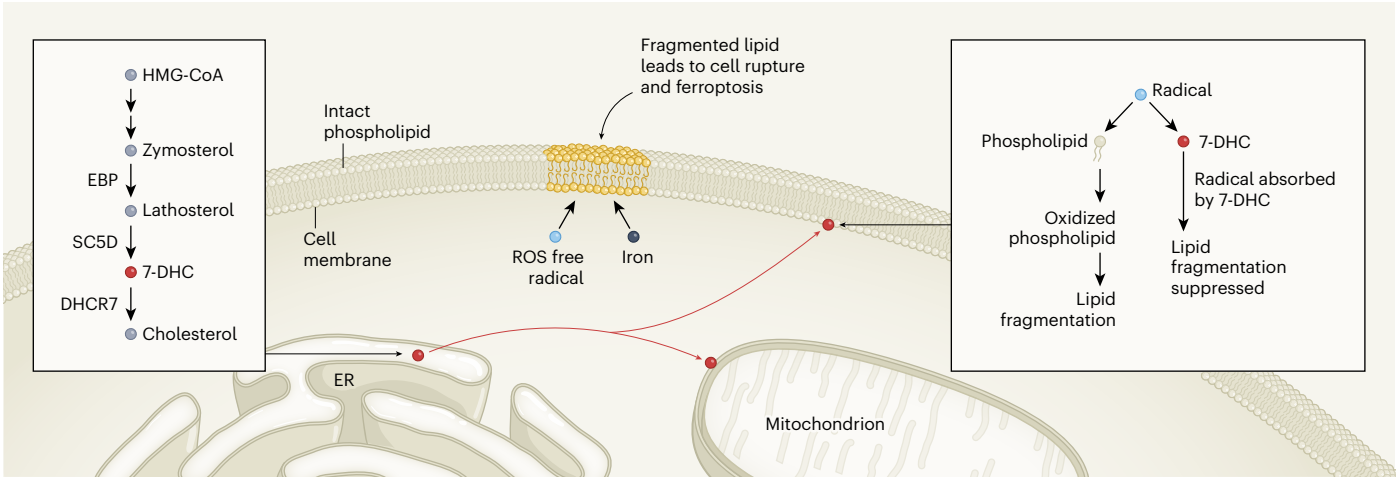

Figure 1 | A molecule that can help to halt cell death by ferroptosis. A hallmark of ferroptosis is lipid modification (oxidation) mediated by iron and molecules called free radicals, such as reactive oxygen species (ROS). Oxidation of lipids, particularly of those called phospholipids, in cellular membranes can kill cells. Freitas *et al.*[1] and Li *et al.*[2] report a previously unknown natural inhibitor of ferroptosis. This molecule, 7-dehydrocholesterol (7-DHC), is made in an organelle called the endoplasmic reticulum (ER) and found on the cell membrane and in another type of organelle, known as mitochondria. 7-DHC is generated in the pathway (not all steps of which are shown) that converts the molecule HMG-CoA to cholesterol through a route that includes the molecules zymosterol and lathosterol and depends on enzymes such as EBP, SC5D and DHCR7. When radicals attack phospholipids, the lipid is oxidized and it fragments. 7-DHC absorbs radicals, counteracting their ability to trigger lipid oxidation and ferroptosis.

The discovery of a natural inhibitor of ferroptosis has profound therapeutic implications. By modulating the levels of 7-DHC, there is the potential to either induce or counteract ferroptosis. Investigating the role of 7-DHC in human cancers, Freitas and colleagues observed that 9.8% of individuals studied who had Burkitt's lymphoma had mutations in the *DHCR7* gene, which encodes the corresponding enzyme. The authors note a less-frequent occurrence of such mutations in people who have a brain cancer called neuroblastoma.

The deletion of *DHCR7* by Li and colleagues increased 7-DHC levels, bolstering the resistance of neuroblastoma cancer cells to ferroptosis when tested *in vitro*. *In vivo* experiments in mice showed that these engineered human neuroblastoma cells evaded ferroptotic death, leading to faster tumour growth, increased tumour spread (metastasis) and decreased survival time compared with results from mice in which the transplanted cells expressed *DHCR7*.

Li *et al.* present evidence of the effect of manipulating 7-DHC levels. They report that inhibiting 7-DHC synthesis by targeting an enzyme called EBP in the cholesterol-synthesis pathway with the molecule TASIN-30 induced ferroptosis. This led to the suppression of tumour growth *in vivo* when human cancer cells with high levels of 7-DHC were injected into mice. It is notable that this cell-death suppression occurred even in the absence of other ferroptosis inducers besides TASIN-30, underscoring the key role of 7-DHC in providing protection against cell death for certain cancer types. This protective mechanism makes these cancer cells susceptible to drugs that inhibit 7-DHC production.

Building on their initial findings, Li and colleagues examined 7-DHC's role in metastasis. The authors hypothesized that before spreading to distant organs, cancer cells need to endure environments that have a condition (oxidative stress) that predisposes the cells to ferroptosis. Li *et al.* used a combination of approaches to investigate this. These included deleting the *DHCR7* gene, inhibiting DHCR7 with the drug AY9944, pretreating melanoma cancer cells with 7-DHC to elevate 7-DHC levels and using TASIN-30 to hinder 7-DHC synthesis. These approaches establish that 7-DHC can fortify melanoma cells, enhancing their survival and accelerating metastasis.

"Harnessing this new understanding with available tools holds the potential to transform clinical treatments."

Using a mouse model of tissue injury, Li and colleagues demonstrated that the accumulation of 7-DHC could be amplified by inhibiting DHCR7, achieved through pre-injury injections of DHCR7 inhibitors. This intervention effectively shielded against injury by preventing ferroptotic death of kidney cells.

The discovery of 7-DHC's role in preventing ferroptosis is a key leap forwards in this area. The therapeutic potential of the finding is strikingly evident. Given the availability of drugs already in clinical use to target DHCR7, harnessing this new understanding with available tools holds the potential to transform clinical treatments for conditions influenced by ferroptosis, if this approach is validated in clinical testing. However, previous research[8,9] indicates that excessive levels of 7-DHC and the products arising from modifications (oxidation) of this molecule might be detrimental, particularly to neuronal and retinal cells. Thoroughly examining the implications for brain and eye health will be crucial before incorporating any 7-DHC-based therapeutic strategies into standard clinical practice.

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