3. Willner, P., D'Aquila, P. S., Coventry, T. & Brain, P. *J. Psychopharmacol.* **9**, 207–213 (1995).
4. Shively, C. A. *Biol. Psychiatry* **44**, 882–891 (1998).
5. Fan, Z. *et al. Cell* **186**, 560–576 (2023).
6. Wang, F. *et al. Science* **334**, 693–697 (2011).
7. Zhou, T. *et al. Science* **357**, 162–168 (2017).
8. Padilla-Coreano, N. *et al. Nature* **603**, 667–671 (2022).
9. Ligneul, R., Obeso, I., Ruff, C. C. & Dreher, J.-C. *Curr. Biol.* **26**, 3107–3115 (2016).
10. Hu, H., Cui, Y. & Yang, Y. *Nature Rev. Neurosci.* **21**, 277–295 (2020).
11. Maier, S. F. & Seligman, M. E. P. *Psychol. Rev.* **123**, 349–367 (2016).
12. Amat, J. *et al. Nature Neurosci.* **8**, 365–371 (2005).
13. Baratta, M. V. *et al. Brain Struct. Funct.* **224**, 1831–1843 (2019).
14. Berton, O. *et al. Science* **311**, 864–868 (2006).
15. Harris, A. Z. *et al. Neuropsychopharmacology* **43**, 1276–1283 (2018).
16. Willmore, L., Cameron, C., Yang, J., Witten, I. B. & Falkner, A. L. *Nature* **611**, 124–132 (2022).
17. Wood, S. K., Walker, H. E., Valentino, R. J. & Bhatnagar, S. *Endocrinology* **151**, 1795–1805 (2010).
18. Stein, A. T., Carl, E., Cuijpers, P., Karyotaki, E. & Smits, J. A. J. *Psychol. Med.* **51**, 1491–1504 (2021).

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Metabolism

Mitochondrial molecule controls inflammation

Taylor A. Poor & Navdeep S. Chandel

Cellular organelles called mitochondria contain their own DNA and RNA. The molecule fumarate has now been found to trigger the release of these nucleic acids into the cytosol, aberrantly activating inflammation. **See p.490 & p.499**

Although mitochondria are often referred to as the powerhouses of the cell, these organelles can also act as signalling hubs that control physiological processes[1]. One such signalling pathway controls inflammation[2]. Now, Zecchini *et al.*[3] (page 499) and Hooftman *et al.*[4] (page 490) report that fumarate (a molecule produced as an intermediate of metabolic processes in mitochondria) triggers the activation of specific inflammation-related pathways. Their findings have implications for cancer and inflammatory diseases.

Viruses that contain double-stranded DNA can be detected in the cell cytosol by the cGAS–STING pathway. By contrast, viral double-stranded RNA in the cytosol is sensed by the RIG-I- and MDA5-dependent mitochondrial antiviral-signalling (RIG-I/MDA5-MAVS) pathway, components of which are anchored to the outer membrane of mitochondria. Both of these pathways lead to the transcription of genes that encode a type of signalling protein called interferon-I (IFN-I), which activates the immune system to mediate antiviral responses.

Notably, mitochondria contain their own circular double-stranded DNA called mtDNA, which is transcribed to mitochondrial RNA (mtRNA) and subsequently translated to produce proteins needed to generate energy-carrying ATP molecules. The release of mtDNA and mtRNA from mitochondria into the cytosol can activate IFN-I-dependent antiviral immunity through the cGAS–STING and RIG-I/MDA5-MAVS pathways[5,6]. This can occur when cells are infected by pathogens such as influenza virus or the bacterium *Mycobacterium tuberculosis*, which can trigger the release of mtDNA. It can also happen during radiation therapy, which generates breaks in mtDNA and so results in the transcription of short mtRNAs that are released into the cytosol.

Zecchini *et al.* and Hooftman *et al.* now reveal that fumarate has a role in mtDNA- and mtRNA-dependent inflammation (Fig. 1). Fumarate is converted to malate by the enzyme fumarate hydratase. Both groups observed that pharmacological or genetic inhibition of fumarate hydratase increased the intracellular levels of fumarate, which activated the RIG-I/MDA5-MAVS pathway.

Zecchini and colleagues found that fumarate also activated the cGAS–STING pathway, although Hooftman *et al.* discovered that inhibiting the activity of STING or silencing the expression of cGAS had no effect on inflammation. The reason for this discrepancy is not fully clear. One simple explanation could be that fumarate has different effects in different cell types, because Zecchini *et al.* used kidney cells, whereas Hooftman *et al.* studied immune cells called macrophages.

How could mtRNA or mtDNA escape from the mitochondrion and pass through the organelle's two membranes into the cytosol? Previous work[6] suggests that BAX and BAK proteins generate pores that release mtRNA, although whether this process involves fumarate is unknown. In addition, mitochondria shed small mitochondrial-derived vesicles

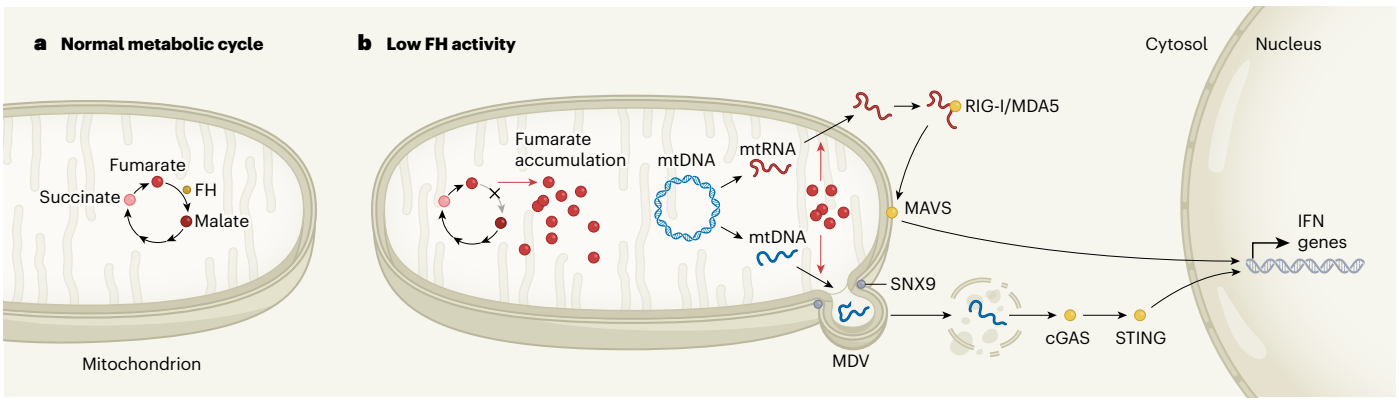

Figure 1 | A role for fumarate in inflammation. a, In organelles called mitochondria, the enzyme fumarate hydratase (FH) converts the molecule fumarate into malate, as part of a normal metabolic cycle. **b**, In some cancers and immune disorders, the activity of FH is reduced, and fumarate accumulates. Two groups[3,4] show that fumarate accumulation leads to the release of nucleic acids from mitochondria. Release of mitochondrial RNA (mtRNA) might involve a change in the electrical potential across the mitochondrial membrane. Mitochondrial DNA (mtDNA) might be released in mitochondrial-derived vesicles (MDVs), which bud off from the membrane in a process that involves the protein SNX9. The released mtRNA and mtDNA activate the RIG-I/MDA5-MAVS and cGAS–STING signalling pathways, respectively. These pathways trigger the transcription of genes that encode type-I interferon (IFN) proteins, which induce inflammation.

(MDVs), which bud from the organelles' membranes[7]. The protein SNX9 facilitates this vesicle-shedding process[8], and decreasing the levels of SNX9 reduces the amount of mtDNA in the cytosol of cells that lack fumarate hydratase activity. Might fumarate induce the release of mtDNA through MDVs?

Zecchini and co-workers propose one explanation for how this pathway might act. Previous research indicates that fumarate can modify proteins by binding to specific cysteine residues — a process called succination[9] — and Zecchini *et al.* demonstrate that fumarate triggers the rapid accumulation of succinated proteins in mitochondria. Perhaps, they suggest, the succination of mitochondrial proteins somehow drives the formation of MDVs. Whether mtRNA could also exit through this pathway remains to be seen.

Hooftman *et al.* put forward an alternative explanation for how nucleic acids escape — that fumarate triggers an increase in the electrical potential across the mitochondrial membrane, which might be linked to the release of mtDNA or mtRNA. They show that increasing the mitochondrial membrane potential causes mtRNA release and induces the expression of interferons in macrophages. Another study[10] has shown that the loss of certain mitochondrial proteins compromises mitochondrial-membrane integrity and internal architecture, leading to an increase in the release of mtDNA. The two groups' hypotheses could fit together, if the mitochondrial proteins required for membrane integrity are succinated by fumarate.

The finding that fumarate accumulation can lead to the release of mtDNA or mtRNA to stimulate interferons has implications for cancer. Mutations that prevent fumarate hydratase from functioning are found in a type of cancer called hereditary leiomyomatosis and renal cell cancer (HLRCC)[11]. The presence of IFN-I signalling is a marker of a 'hot' tumour (one that has been infiltrated by immune cells), and immune infiltration is frequently observed in HLRCCs that involve the mutation of fumarate hydratase[12]. Immune infiltration correlates with responsiveness to immunotherapy. It will therefore be interesting to see whether invoking the release of mtDNA and mtRNA therapeutically could elicit immune infiltration to improve how a cancer responds to immunotherapy.

There are implications for inflammatory diseases, too. For instance, the autoimmune disease systemic lupus erythematosus (SLE) is characterized by high levels of IFN-I-dependent inflammation and an increase in circulating mtDNA in the blood plasma[13]. Hooftman *et al.* found that the activity of fumarate hydratase was suppressed in the blood of people with SLE compared with healthy individuals, but it remains to be seen whether the reduction in fumarate hydratase activity is responsible for inflammation in these people.

Together, these two studies expand our understanding of how the mitochondria mediate immune responses. Future research should examine whether fumarate accumulation might drive any of the numerous diseases associated with IFN-I-dependent inflammation — from SLE to genetic diseases such as Aicardi–Goutières syndrome. Biologists will also undoubtedly ask whether fumarate levels correlate with immune infiltration in other hot tumours.

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1. Chakrabarty, R. P. & Chandel, N. S. *Biochemistry* **44**, 2–8 (2022).
2. Marchi, S., Guilbaud, E., Tait, S. W. G., Yamazaki, T. & Galluzzi, L. *Nature Rev. Immunol.* **23**, 159–173 (2023).
3. Zecchini, V. *et al. Nature* **615**, 499–506 (2023).
4. Hooftman, A. *et al. Nature* **615** 490–498 (2023).
5. West, A. P. *et al. Nature* **520**, 553–557 (2015).
6. Tigano, M., Vargas, D. C., Tremblay-Belzile, S., Fu, Y. & Sfeir, A. *Nature* **591**, 477–481 (2021).
7. Sugiura, A., McLelland, G.-L., Fon, E. A. & McBride, H. M. *EMBO J.* **33**, 2142–2156 (2014).
8. Todkar, K. *et al. Nature Commun.* **12**, 1971 (2021).
9. Zheng, L. *et al. Nature Commun.* **6**, 6001 (2015).
10. He, B. *et al. Cell Rep.* **41**, 111774 (2022).
11. Yong, C., Stewart, G. D. & Frezza, C. *Nature Rev. Nephrol.* **16**, 156–172 (2020).
12. Dong, P. *et al. Clin. Cancer Res.* **28**, 4807–4819 (2022).
13. Giaglis, S. *et al. RMD Open* **7**, e002010 (2021).

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Tumour biology

Bleb protrusions help cancer cells to cheat death

Michal Reichman-Fried & Erez Raz

Processes that regulate cell death can rid the body of cancer cells. However, some of these cells have ways to thwart such processes, and one such death-defying mechanism has been found to rely on cellular protrusions called blebs. **See p.517**

If cells do not attach properly to their external environment, this typically triggers a regulated process of cell death that helps to maintain tissue integrity and removes potentially harmful cells[1,2]. Cancer cells can develop the ability to exist without being attached to other tissues, which is a key feature enabling their survival and spread[1]. Weems *et al.*[3] report on page 517 the discovery of a mechanism that enables detached malignant cells to survive — one that depends on cellular protrusions named blebs[4].

Regulated cell death requires the activation of signalling pathways that induce cell elimination[1], and cells can evade this process by activating opposing signals that promote survival. In the system that Weems and colleagues studied, cell death is triggered by a loss of attachment to surrounding tissues, in a process termed anoikis[1,2]. Thus, although some cell types, such as blood cells, can live and perform their functions in the absence of strong and stable adhesion to their surroundings[5], detached cells typically undergo anoikis.

The survival of cells normally eliminated by anoikis can lead to adverse clinical consequences, such as the persistence and spread (metastasis) of cancer cells[6]. Therefore, identifying mechanisms that control the balance between cell survival and death after detachment should help in understanding the progression and successful implementation of normal physiological events, and might also shed light on the basis of some diseases.

Cells that migrate and invade tissues under low-adhesion conditions often form blebs (Fig. 1), which are spherical cellular protrusions that are powered by hydrostatic pressure[4,5,7]. In the case of tumour formation and metastasis, the presence of blebs is correlated with tumour invasiveness and the activation of pro-survival pathways[7,8]. Interestingly, on detachment, metastatic cells from the skin cancer melanoma form blebs, but, unlike most non-malignant cells, they do not undergo anoikis. These observations prompted Weems and colleagues to explore the mechanisms by which melanoma cells evade death in the absence of adhesion, focusing on a possible role for blebs.

The authors showed that inhibiting the formation of blebs meant that melanoma cells lost their ability to evade anoikis. Intriguingly, under conditions that facilitated bleb growth,