TUMOUR BIOLOGY

Survival skills ensure that cancer spreads

How cancer cells migrate to a secondary site and become established there is not fully understood. An analysis of mouse and human cancer cells could help settle the debate about the role of the protein E-cadherin in this process. SEE LETTER P.439

ROGER R. GOMIS

When cancer spreads from its primary site to become established at a secondary location, this process, termed metastasis, is often fatal. The timing of metastasis initiation varies depending on the size, stage and differentiation status of the tumour at the primary site[1]. Metastasis probably requires cancer cells to undergo changes, including those that promote the acquisition of invasive properties. Loss of expression of the protein E-cadherin enables cells to migrate, but how E-cadherin fulfils its role as a central regulator of metastasis isn't fully understood[2]. On page 439, Padmanaban *et al.*[3] report that E-cadherin contributes to an aspect of metastasis that differs from the protein's previously known effect on cell invasion and migration.

E-cadherin is present on the membrane of epithelial cells, which form a barrier layer on surfaces of the body. When a process called epithelial-to-mesenchymal transition (EMT) is triggered, loss of E-cadherin occurs, and epithelial cells acquire the characteristics of mesenchymal cells, which are highly mobile. EMT is usually activated by specific stimuli[4], such as exposure to the signalling protein TGF-β. The process occurs in embryonic development[5], and aids the repositioning of normal epithelial cells within organs during healing[6]. However, EMT can be hijacked for the development and spread of cancer[7–11].

Adhesion between epithelial cells is mediated by E-cadherin, leading to the view that such adhesion suppresses metastasis[12]. Yet, counter-intuitively, some pieces of the puzzle do not fit this model. There is compelling clinical evidence that metastatic cancer cells commonly express E-cadherin and molecules associated with epithelial-cell fate[13]. For example, E-cadherin is found in metastatic cells in a type of breast cancer called invasive ductal breast carcinoma[14,15]. One idea to reconcile this discrepancy is if metastatic cancer cells that have undergone EMT and reached a secondary site then undergo a reversal process called mesenchymal-to-epithelial transition[16]. The presence of E-cadherin in cells at a secondary site does not then necessarily indicate that the protein helped metastatic cells to become established there.

Padmanaban and colleagues investigated E-cadherin's role in a broad spectrum of mouse models of different types of invasive ductal breast carcinoma, and also analysed human cancer cells that were introduced into the mouse models. The authors engineered mouse or human cancer cells so that E-cadherin expression could be lowered or blocked and its expression tracked by monitoring fluorescent proteins. Cancer cells that expressed E-cadherin displayed less migratory behaviour *in vitro* than did those that did not express it, consistent with previous findings.

When the authors studied the behaviour of human cancer cells transplanted into mice, they found that, unexpectedly, cells expressing E-cadherin were more common than those lacking E-cadherin in primary tumours. This was also the case in tumour cells that had escaped the primary site, termed circulating tumour cells (CTCs), and in tumours that had metastasized. And when tumour cells were implanted in the animals' mammary gland or injected into the bloodstream, those that expressed E-cadherin became established at a secondary site, whereas those lacking E-cadherin rarely did so (Fig. 1). This is surprising, because E-cadherin has not previously been shown to have a role in aiding the survival of metastatic cancer cells.

The authors found that, compared with E-cadherin-expressing cancer cells, cells lacking E-cadherin had higher levels of expression of genes associated with a type of cell death called apoptosis, and of genes that function in stress-related pathways. TGF-β and molecules known as reactive oxygen species (ROS) contributed to the upregulation of these pathways. Padmanaban *et al.* carried out *in vitro* and *in vivo* experiments in which they used inhibitors to target TGF-β, ROS or components required for apoptosis, and found that this treatment counteracted the effects of E-cadherin loss in cancer cells. Pathways involving TGF-β and ROS are known to be important in triggering apoptosis in invasive ductal breast carcinoma cells that have low levels of E-cadherin expression as they start to undergo EMT[14,17].

Padmanaban and colleagues have uncovered a way in which E-cadherin functions in a context-dependent manner to promote tumour progression and metastasis by helping metastatic cells to overcome cellular stress mediated by TGF-β and ROS. E-cadherin loss compromised the metastatic potential of the cells by affecting cell survival and thereby impairing

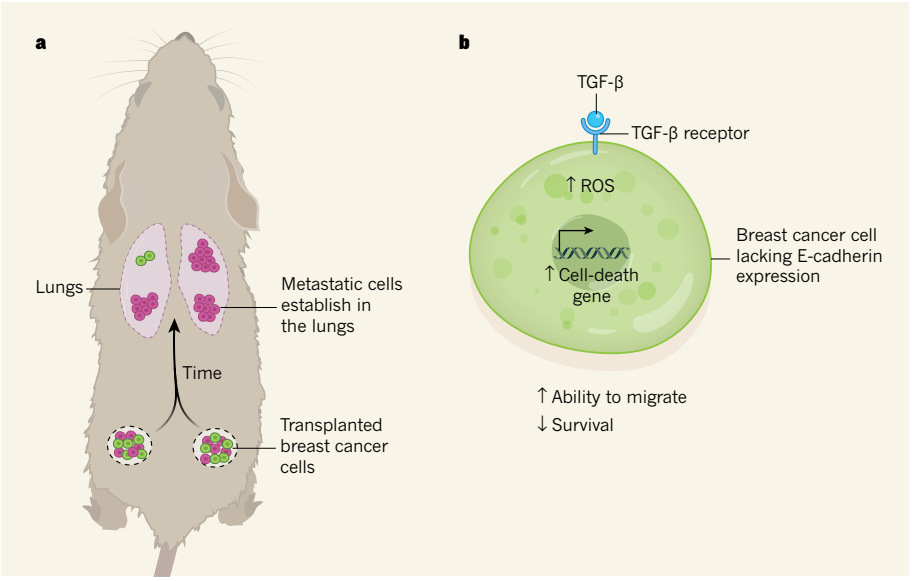

Figure 1 | E-cadherin expression boosts the establishment of cancer at a secondary site. a, Padmanaban *et al.*[3] report an analysis of factors affecting the ability of mammalian (mouse or human) breast cancer cells to become established at a secondary site through a process called metastasis. The authors engineered breast cancer cells to express (pink) or lack expression of (green) the protein E-cadherin, and transplanted these cells into mouse mammary glands. The tumour cells that expressed E-cadherin became established in the lungs more often than did those that lacked expression of E-cadherin. **b**, If breast cancer cells lacked E-cadherin, both the levels of molecules called reactive oxygen species (ROS) and expression of genes associated with cell-death processes were increased in these cells through pathways triggered by the protein TGF-β and its receptor, compared with their levels in cells expressing E-cadherin. Such cells had a higher than normal ability to migrate, but a lower than normal ability to survive.

tumour establishment and cell proliferation at a secondary site. Thus, with regard to the metastasis of invasive ductal breast carcinoma, the pro-survival contribution of E-cadherin outweighs the advantage of E-cadherin loss boosting invasiveness.

A future research direction worth pursuing would be to determine whether there are any differences in the expression of the gene that encodes E-cadherin in cells from primary tumours, CTCs and metastatic sites. During EMT, cells are thought to go through distinct states[18], but the cell-fate transitions that occur in tumours undergoing EMT are still unknown, and might vary depending on the tumour type. It is therefore unclear whether invasive ductal breast carcinoma cells that express E-cadherin, even at low levels, are in an EMT state or are a specific cellular lineage that is not undergoing EMT. Single-cell RNA sequencing could shed light on this by revealing whether there are distinct cell populations (clones) in primary tumours or metastatic cells that do not show signs of transitioning through EMT states.

Collective dissemination, in which different types of cell migrate together in a cluster, boosts a tumour's ability to colonize distant sites[19]. If such tumour co-dependencies occur between stress-resistant cells that have high levels of E-cadherin and invasive cells that have low E-cadherin levels, collective dissemination might aid metastasis after a person's tumour has been treated by, for example, chemotherapy, causing cellular stress. Examining the patterns of such tumour evolution, as well as analysing the mechanisms of therapy failure and pathways of cancer-cell growth, particularly of treatment-resistant cancer cells, will be crucial for the development of new clinical targets, treatments and therapeutic windows of opportunity. Whether E-cadherin expression has a key role in the survival of different types of tumour or in different forms of metastasis should also be explored.

The general factors that affect tumour growth provide clues to why cellular adaptations occur during metastasis. In-depth analyses are nevertheless needed, because the genetic background of a given type of tumour might affect the requirements for metastasis to occur. Padmanaban *et al.* have shown how E-cadherin is needed for metastasis of invasive ductal carcinoma, but other types of cancer might use alternative mechanisms to manage stress, perhaps generating different tumour vulnerabilities that could be exploited therapeutically.

It would be better to prevent metastasis than to have to treat cells that have metastasized. Understanding how E-cadherin expression is stabilized might reveal a vulnerability of cancer cells on a path towards metastasis. Developing tailored treatments to tackle or prevent metastasis should be a goal of cancer research, and we are headed in the right direction to make progress on this front. ■

Roger R. Gomis *is in the Oncology Program, Institute for Research in Biomedicine, Barcelona Institute of Science and Technology, Barcelona 08028, Spain, and at CIBERONC, Barcelona, Spain, at the University of Barcelona, and at the Catalan Institution for Research and Advanced Studies, Barcelona.*
e-mail: roger.gomis@irbbarcelona.org

1. Kennecke, H. *et al. J. Clin. Oncol.* **28**, 3271–3277 (2010).
2. Bogenrieder, T. & Herlyn, M. *Oncogene* **22**, 6524–6536 (2003).
3. Padmanaban, V. *et al. Nature* **573**, 439–444 (2019).
4. Yang, J. & Weinberg, R. A. *Dev. Cell* **14**, 818–829 (2008).
5. Nakaya, Y. & Sheng, G. *Dev. Growth Differ.* **50**, 755–766 (2008).
6. Haensel, D. & Dai, X. *Dev. Dyn.* **247**, 473–480 (2018).
7. Aiello, N. M. & Kang, Y. *J. Exp. Med.* **216**, 1016–1026 (2019).
8. Onder, T. T. *et al. Cancer Res.* **68**, 3645–3654 (2008).
9. Frixen, U. H. *et al. J. Cell Biol.* **113**, 173–185 (1991).
10. Derksen, P. W. B. *et al. Cancer Cell* **10**, 437–449 (2006).
11. Ciriello, G. *et al. Cell* **163**, 506–519 (2015).
12. Onder, T. T. *et al. Cancer Res.* **68**, 3645–3654 (2008).
13. Chaffer, C. L. *et al. Cancer Res.* **66**, 11271–11287 (2006).
14. Acs, G., Lawton, T. J., Rebbeck, T. R., LiVolsi, V. A. & Zhang, P. J. *Am. J. Clin. Pathol.* **115**, 85–98 (2001).
15. Kowalski, P. J., Rubin, M. A. & Kleer, C. G. *Breast Cancer Res.* **5**, R217–R222 (2003).
16. Pastushenko, I. & Blanpain, C. *Trends Cell Biol.* **29**, 212–226 (2019).
17. Zhang, Z. *et al. Cell Death Dis.* **9**, 83 (2018).
18. Pastushenko, I. *et al. Nature* **556**, 463–468 (2018).
19. Aceto, N. *et al. Cell* **158**, 1110–1122 (2014).

This article was published online on 4 September 2019.

ASTROPHYSICS

A galaxy with hiccups

A galaxy has been seen producing strong, regular bursts of X-rays that recur on timescales of hours. The eruptions imply that the matter flowing onto the galaxy's central black hole undergoes repeated restructuring. SEE LETTER P.381

BOŻENA CZERNY

On page 381, Miniutti *et al.*[1] report a stunning phenomenon unlike anything observed before in a galaxy: almost-periodic eruptions that increase the X-ray emission from the host galaxy by a factor of up to 100, and which last only about an hour. Even more remarkably, these galactic hiccups recur on timescales of hours — raising questions about the mechanism that could produce such rapidly repeating events.

The galaxy in question is called GSN 069, and has a past record of surprising behaviour. It was initially classified as a field galaxy[2] (a galaxy that does not belong to a larger group or cluster of galaxies) in 1989, and at first was not observed to produce any unusual emission. But then, in 2010, its X-ray emission brightened[3] by a factor of at least 240.

This event identified GSN 069 as an 'active' galaxy, in which the efficient inflow of plasma onto a central supermassive black hole causes enormous amounts of energy to be dissipated as the material approaches the black hole's event horizon (the boundary beyond which nothing can escape the black hole's gravitational field). Some of the dissipated energy leads to the strong emission of electromagnetic radiation. Because the inflow is turbulent, the emission from the central regions of active galaxies is variable. The variability is usually stochastic, with continuous irregular increases and decreases in brightness at all timescales — which, in the case of GSN 069, were superimposed on an overall dimming trend[1].

However, in late 2018, Miniutti *et al.* observed variations in the emission from GSN 069 that were nothing like the pattern described above. They detected two X-ray eruptions by chance on 24 December 2018, during a half-day observation period using the XMM-Newton space telescope. The X-ray emission unexpectedly brightened by a factor of more than 30 over the course of about 30 minutes, returned to its previous state in about another 30 minutes, and then erupted again after a further 8 hours. The authors recorded five more eruptions during 16 and 17 January 2019, using the same instrument (Fig. 1), and three more during 14 and 15 February, using the space-based Chandra X-ray Observatory. These eruptions were extremely regular, separated in time by about 9 hours, and each lasted just over an hour.

What could the explanation be? The radiation spectra produced by many active galaxies have been interpreted as coming from a complex flow of material onto a black hole[4,5] — that is, a flow in which most of the material comes from a relatively cold (about 100,000 kelvin) disk of matter, but some of which comes from a corona of less-dense material that surrounds the disk and is about ten times warmer. In the case of the eruptions observed by Miniutti and co-workers, most of the energy must be released from a region close to a black hole that is compact enough to undergo coordinated changes of state.

Some hints about the eruption source in GSN 069 come from details of Miniutti and co-workers' analysis of how the amplitude of the outbursts depends on the range of energies of the studied photons. The