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METASTASIS

Pushing through a tough stretch

When cells from a primary tumour enter the bloodstream, they are disseminated throughout the body and eventually land in the microvasculature of secondary organs. There, they are deformed within capillaries of small diameter, where up to 90% of the cancer cells die, as they are unable to survive under such mechanical stress. However, 10% of cells manage to endure this stress. Tavazoie and colleagues have now unveiled the mechanism by which such cells survive microvascular deformation at the target organs and subsequently infiltrate the parenchyma and form metastases.

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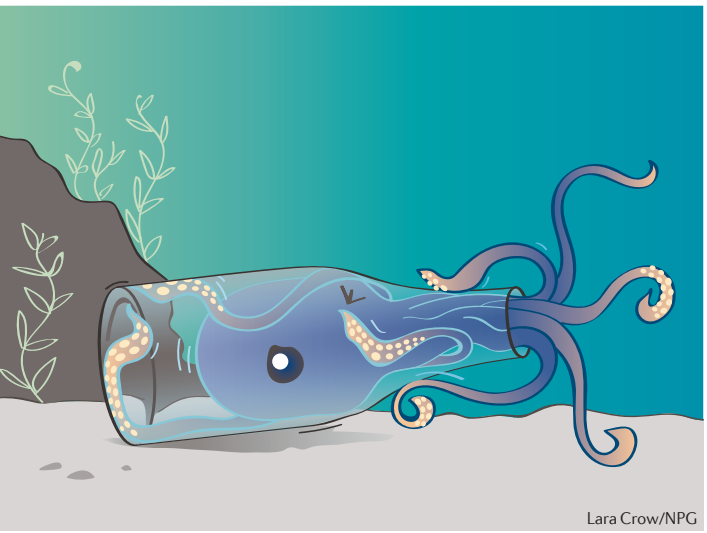
The authors carried out whole-transcriptome RNA sequencing both of metastatic sub-clones (CN-LM1A and MDA-LM2) from human breast cancer cell lines selected *in vivo* by Massagué and colleagues in immunodeficient mice and of the CN34 and MDA-MB-231 parental lines from which they were derived. Five genes were expressed in the transcriptomes of both metastatic sub-lines at frequencies higher than those in the respective — and less metastatic — parental populations; one of these genes encoded the nonsense variant *PANX1-C268T*, which was significantly enriched in both sub-lines. The mutation encoding *PANX1-C268T* substitutes a premature termination codon in the transmembrane channel protein pannexin 1 (PANX1) — which mediates the release of ATP from cells into the extracellular space — thereby generating a shorter variant of PANX1, which the authors called $PANX^{11-89}$. This mutant was also able to enhance the release of ATP but only when co-expressed with wild-type full-length PANX1. Indeed, highly metastatic CN-LM1A and MDA-LM2 sub-lines secreted nearly fivefold higher amounts of ATP in a PANX1-dependent manner than their less metastatic parental cell populations, without expressing higher levels of PANX1.



Lara Crow/NPG

Next, the authors sought to investigate the extent to which PANX1 channels were responsible for the metastatic potential of breast cancer cells. They injected human breast cancer cells into the tail of immunodeficient mice and quantified the extracellular ATP released from these cells, observing substantial extracellular ATP signal in the lungs that was attenuated when the cells were pretreated with the PANX1 inhibitor carbenoxolone (CBX). Inhibition of PANX1 also inhibited metastasis formation, although it did not inhibit proliferation, invasion, transendothelial migration or anchorage-independent cell survival capacity. This suggested that PANX1 inhibition increases cell death within the microvasculature and, therefore, that PANX1 channel activity increases the metastatic capacity of breast cancer cells by promoting survival in the microvasculature.

The authors observed that at the time when PANX1 activity promoted survival, cancer cells were confined to the pulmonary microvasculature and had adopted a morphologically elongated shape. To determine whether PANX1 promotes cell survival during cell deformation, the authors carried out hypotonic cell swelling experiments — in which the plasma membrane is stretched — and observed that PANX1-mediated ATP release in CN-LM1A and MDA-LM2 cells was dramatically increased and that these cells were more resistant than the parental cell lines to lethal stretch. ATP release stimulated by plasma membrane stretch subsequently activated P2Y purinergic receptors, inducing a signalling cascade that inhibited apoptosis and ultimately protected cancer cells from lethal mechanical injury.

Further experiments revealed that $PANX^{11-89}$ activates PANX1 channels and that treatment of CN-LM1A cells and other highly metastatic cells with CBX — which has been approved for the treatment of gastric reflux — inhibited the ability of these cells to colonize distant organs such as the lungs.

Future studies will reveal the signal transduction mechanism that mediates activation of PANX1 and cell survival, but for now, PANX1 channels seem to be a potential target in the prevention of metastatic disease.

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ORIGINAL RESEARCH PAPER Furlow, P. W. *et al.* Mechanosensitive pannexin-1 channels mediate microvascular metastatic cell survival. *Nat. Cell Biol.* **17**, 943–952 (2015)