*Nature Reviews Cancer* | https://doi.org/10.1038/s41568-018-0089-1 | Published online 3 December 2018

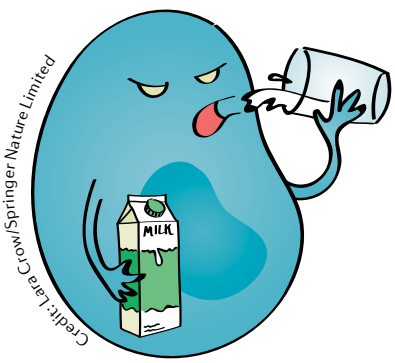
METASTASIS

# A source of calcium

> Cx43 expression in bone lesions of BrCa clinical samples was highest among all sites of metastases

Breast cancer (BrCa) most frequently metastasizes to bone, often after long latency, suggesting that metastatic seeds are resistant to therapy and can regrow. Although in overt bone metastases targetable signalling pathways between osteoclasts and cancer cells that drive growth are known, it is less clear which mechanism mediates early-stage bone colonization. Wang et al. have identified calcium flux as a mechanism of crosstalk between the osteogenic niche and cancer cells, which promotes progression of bone micrometastases.

Performing gene set analysis of a published data set of metastasized BrCa, they identified the transcription factors nuclear factor of activated T cells (NFAT) and myocyte-specific enhancer factor 2 (MEF2), which are downstream of calcium signalling, as more highly active in bone metastases than in other metastases of BrCa. A mouse model generated through intra-iliac artery (IIA) injection of MCF7 BrCa cells confirmed upregulation of NFAT and MEF2 transcriptional activities in bone metastases compared with the orthotopic tumour. A similar finding was obtained with 3D co-culture of MCF7 cells with osteogenic cells (mesenchymal stem cells (MSCs) or osteoblasts (OBs)), in which cancer cell proliferation was increased relative to mono-cultured MCF7 cells.




Credit: Lara Cowl/Springer Nature Limited

Calcineurin (CaN) and $Ca^{2+}$/calmodulin-dependent protein kinase type II (CaMKII) are transducers of calcium signalling upstream of NFAT and MEF2. In a bone-in-culture array (BICA), in which cancer cell-harbouring bone tissue segments are cultured ex vivo, dominant negative CaN (DN-CaN) or DN-CaMKII, alone or combined, decreased the growth of BrCa cells compared with vector controls. IIA-injected MCF7 cells expressing DN-CaN or DN-CaMKII colonized bone less in mice, compared with MCF7 cells expressing the control vectors.

When MCF7 cells were co-cultured with OBs in $Ca^{2+}$-containing media, intracellular $Ca^{2+}$ levels were higher than when cultured alone or when cultured with OBs in $Ca^{2+}$-free media, suggesting unidirectional transfer of $Ca^{2+}$ from OBs to MCF7 cells. This transfer occurred directly through gap junctions and increased calcium signalling in cancer cells. IIA-injected MCF7 cells in bone lesions had upregulated expression of connexin 43 (Cx43), the main component of gap junctions in bone, compared with cells in the orthotopic tumour. Cx43 expression in bone lesions of BrCa clinical samples was highest among all sites of metastasis. Bone colonization of IIA-injected MCF7 cells expressing DN-Cx43 or treated with the gap junction inhibitor carbenoxolone (CBX) was delayed compared with the control conditions. Also, spontaneous bone lesions in a syngeneic orthotopic mouse model of BrCa (4T1.2 model) were reduced in mice treated with CBX. However, the long-term treatment of mice with CBX caused substantial side effects, which is why alternative strategies were sought, focusing on calcium signalling and/or mTOR signalling (which the authors had previously shown promoted bone metastasis).

Using the BICA to screen 68 small-molecule epigenetic modulators as well as 63 FDA or foreign administration-approved antineoplastic drugs, the authors identified arsenic trioxide ($As_2O_3$) and danusertib, both of which decreased bone lesions in the 4T1.2 model but did not affect primary tumour growth and had low toxicity. Both agents decreased NFAT activity and $Ca^{2+}$ concentrations in cancer cells when co-cultured with OBs. Whereas only danusertib also reduced mTOR signalling, only $As_2O_3$ led to reduced expression of the gene encoding Cx43. Indeed, expression of constitutively active CaMKII in MCF7 cells abolished the ability of $As_2O_3$ to reduce bone colonization in vivo compared to control. To model late-onset bone metastasis, the researchers lowered the levels of oestrogen that mice received in their diet. In those mice, IIA injection of MCF7 cells did not lead to orthotopic tumour growth, but still induced bone colonization. $As_2O_3$ treatment reduced the number of mice with bone colonization and increased relapse-free survival.

$As_2O_3$ is in clinical use for the treatment of acute promyelocytic leukaemia and should be further pursued in studies for potential use in patients at risk of bone metastases.

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**ORIGINAL ARTICLE** Wang, H. et al. The osteogenic niche is a calcium reservoir of bone micrometastases and confers unexpected therapeutic vulnerability. *Cancer Cell* **34**, 823–839 (2018)