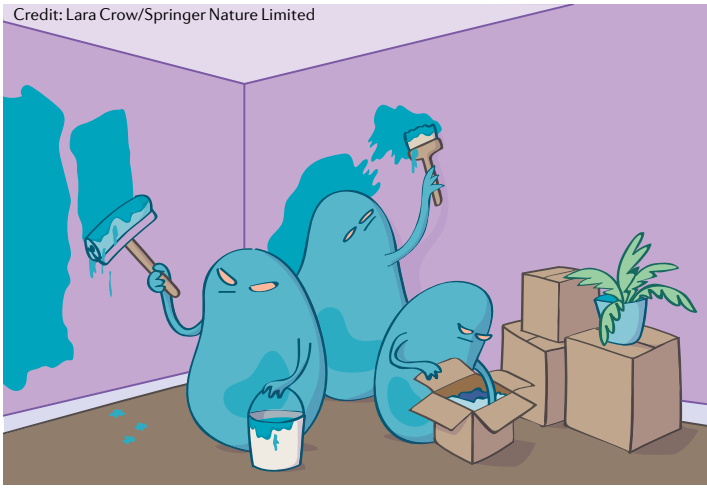
TUMOUR METABOLISM

Renovation in progress


Credit: Lara Crow/Springer Nature Limited

Cancer cells modify the metastatic niche to promote growth by remodelling extracellular matrix (ECM). The differences in nutrient availability and metabolic reactions between tissues may determine the ability of cancer cells to metastasize. Elia et al. identified that metastatic breast cancer cells use pyruvate, abundant in lung tissue, to drive collagen-based ECM remodelling in the lung metastatic niche.

The authors created in vitro conditions in which cancer cells are forced to produce ECM to enable 3D growth in spheroids by culturing cancer cells on soft agar coated plates. When different nutrients were omitted from the culture media, only the depletion of pyruvate reduced growth of *RAS*-transformed breast epithelial cells (MCF10A-*H-RAS*^{V12}), and the reduced growth was rescued by ECM supplementation with matrigel. Collagen, the most abundant protein in the ECM, undergoes hydroxylation before being secreted. In human (MCF10A-*H-RAS*^{V12}, MCF7 and HCC70) and mouse (4T1 and EMT6.5) breast cancer cells, collagen synthesis and hydroxylation was promoted by pyruvate in the media and decreased when monocarboxylate transporter 2 (MCT2), which enables pyruvate uptake, was depleted through CRISPR-mediated knockout (*MCT2* KO) compared with controls. By contrast, collagen synthesis or hydroxylation in non-tumorigenic MCF10A cells were not sensitive to pyruvate depletion.

Collagen hydroxylation leads to increased collagen stability, which in turn impairs digestion by matrix metalloproteinase 8 (MMP8). In an assay measuring MMP8-mediated digestion, pyruvate depletion led to increased digestion of hydroxylated collagen in MCF10A-*H-RAS*^{V12} and 4T1 cells compared with cells cultured in fully supplemented media, showing that pyruvate promotes collagen stability through hydroxylation.

Pyruvate can contribute to intracellular abundance of α-ketoglutarate (αKG), citrate and malate, all levels of which were reduced when pyruvate was depleted in breast cancer cells. Importantly, only supplementation with αKG restored levels of hydroxylated collagen and collagen stability in the absence of pyruvate. Pyruvate contributes to αKG production through a reaction catalysed by alanine aminotransferase (ALT). This reaction converts pyruvate into alanine, while simultaneously converting glutamate into αKG. Accordingly, more carbons derived from pyruvate were found in alanine than in αKG, which consisted of carbons derived from the glutamate precursor glutamine. Also, inhibition of ALT2 reduced the abundance of αKG as well as the levels of hydroxylated collagen in the presence of pyruvate, supporting the link between pyruvate and αKG in promoting collagen hydroxylation.

The enzyme responsible for collagen hydroxylation, prolyl 4-hydroxylase subunit α1 (P4HA1), uses αKG as a co-substrate that is converted into succinate. The addition of succinate to the media, which as the end product of the above reaction could inhibit the P4HA1 activity, decreased the levels of hydroxylated collagen in breast cancer cells significantly, whereas subsequent addition of αKG restored them, indicating that pyruvate can promote P4HA1 activity by providing αKG as a substrate.

In tumours and metastases initiated through orthotopic or intravenous injections of 4T1 or EMT6.5 breast cancer cell lines into BALB/c mice, the researchers studied pyruvate uptake in vivo. Pharmacological or genetic inhibition was achieved by injection of the MCT inhibitor α-cyano-4-hydroxycinnamic acid (αC4HA) or CRISPR-mediated *Mct2* KO, respectively. Confirming observations in vitro, αC4HA or *Mct2* KO decreased levels of pyruvate and pyruvate-derived metabolites in the blood plasma and in lung metastases of both models compared with controls. αC4HA or *Mct2* KO also decreased the levels of hydroxylated collagen and deposition of functional collagen in the lung metastatic niche. In mice treated with αC4HA these effects were restored by intraperitoneal injection of αKG.

> “supplementation with αKG restored levels of hydroxylated collagen”

In both models, the growth of the primary tumour was unchanged in αC4HA-treated mice or *Mct2*-KO mice compared with the respective controls. However, αC4HA or *Mct2* KO decreased the metastatic burden in the lungs of both models compared with the respective controls. Importantly, treatment of mice that received αC4HA with αKG restored the number of metastases to the level seen in mice that received the vehicle control. Similarly, short hairpin RNA-mediated knockdown of ALT2 in 4T1 or EMT6.5 cells reduced metastatic burden upon orthotopic injection into mice.

If growth of breast cancer metastases in the lungs of patients is similarly supported by this metabolite-dependent regulation of ECM remodelling, pyruvate uptake and the ALT reaction might present a new targeting opportunity.

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