METASTASIS

Adapting to change

The lymphatic system enables tumour cell metastatic spread into distant lymph nodes (LNs) and organs. The presence of tumour cells in the draining LN is therefore a predictor for metastasis and mortality of patients with cancer. With the LN posing a microenvironment distinct from the primary tissue, an adaptation of disseminating tumour cells to this change is necessary to enable metastatic spread. Lee et al., who present their findings in *Science*, have identified how tumour cells adapt to the fatty acid-rich environment in LNs, by activation of YAP signalling.

Initial transcriptomic analysis compared three sequential stages of melanoma metastasis into the draining LN, using the B16F10 melanoma mouse model with footpad implantation of green-fluorescent protein-tagged melanoma cells. This analysis revealed upregulation of lipid metabolism pathways in LN micrometastasis and macrometastasis, in particular fatty acid oxidation (FAO) and peroxisome proliferator-activated receptor-α signalling. In line with this, LN metastatic tumours accumulated more ^{14}C-labelled oleic acid than primary tumours in vivo. Melanoma cells derived from the primary tumour or melanoma cells that underwent several rounds of isolation from LN metastasis and inoculation into the footpad, thereby creating a LN-metastasis-prone cell population, both had higher levels of oxidation of fatty acids compared with glucose or glutamine in vitro. In addition, ex vivo oxidation of ^{14}C-labelled palmitic acid in LN metastatic tumours reached levels similar to that of brown adipose tissue, and higher than that in primary tumours or distant metastasis in the lung. Supporting the essentiality of FAO for metastatic growth in LNs, the FAO inhibitor etomoxir reduced LN metastasis in the melanoma mouse model, without affecting the growth of the primary tumour or the size of the LN, compared with untreated melanoma-bearing mice.

Among the differentially regulated oncogenic signalling pathways in LN metastasis, the upregulation of the transcriptional co-activator YAP and its targets stood out as the mediator of FAO activation. Among the candidate genes, only knockdown of *Yap* reduced FAO in metastasis-adapted cells, and overexpression of YAP increased FAO levels and the growth of B16F10 cells in the LN. Importantly, nuclear localization of YAP, indicative of its activation, was mainly found in tumour cells at the invasive front of LN metastasis, whereas YAP in primary tumour cells or in lung metastatic tumour cells was mainly cytoplasmic. These findings were replicated in two different breast cancer mouse models. Accordingly, when YAP was depleted in mice with metastatic melanoma through doxycyline-inducible knockdown, LN metastasis was reduced, and primary tumour growth or lung metastasis were unchanged compared with untreated controls. Also, when YAP-depleted melanoma or 4T1 breast cancer cells were directly injected into LNs, growth was delayed.

To find out how YAP was activated in LN-residing tumour cells, the researchers focused on potential signalling ligands present in this environment. Bile acids, required for dietary fat digestion, but also known activators of YAP, were increased in LN-metastatic melanoma and in systemic lymph compared to normal tissues or lymph of healthy mice. Indeed, treatment with the bile acid taurodeoxycholic acid (TDCA) induced YAP activation in melanoma cells in vitro and promoted growth of LN-metastatic melanoma compared with controls. Cholesterol is a precursor for bile acids, and cholesterol levels were similar in tumour tissues compared with normal tissues. However, depletion of cholesterol 7 α-hydroxylase, which converts cholesterol into bile acids, in B16F10 melanoma cells reduced their metastatic growth in LNs compared with wild-type cells, suggesting that melanoma cells could contribute to the production of bile acids in the LN. However, this possibility requires further investigation. In patients with melanoma, approximately half of dissected metastatic LNs had predominantly nuclear YAP expression, which correlated with reduced metastasis-free survival, pinpointing YAP as a potential biomarker for metastasis.

Credit: Lara Crow/Springer Nature Limited

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Naive LNs have higher levels of fatty acids compared with footpad tissue in mice. The metabolic plasticity of melanoma cells enables their metastatic growth in this environment — cells spreading from the footpad adapt to the LN environment through bile acid-mediated activation of YAP and YAP-dependent FAO. Yet, the mechanisms by which YAP regulates FAO and by which FAO enables tumour growth in this context are unclear. In addition, whether tumour cells circulating in the lymph are primed for growth in fatty acid-rich environments in response to circulating bile acids will be interesting to investigate.

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