METASTASIS

Breaching barriers

> “patients with leptomeningeal metastasis had the highest levels of C3 in the CSF”

Leptomeningeal metastasis is the spread of cancer cells into the leptomeninges, the two innermost layers of tissue that surround the brain and spinal cord and through which the cerebrospinal fluid (CSF) circulates. This complication of cancer currently occurs in approximately 5–10% of patients with solid tumours and is often terminal only weeks after diagnosis in untreated patients. Mechanistically, little is known about how cancer cells can breach the blood–CSF barrier and once there, how they survive in the acellular, nutrient-poor environment of the CSF. Now, Boire *et al.* have established that cancer cells can adapt to and grow in this unique metastatic environment by modifying the CSF by secretion of complement component 3 (C3).

Leptomeningeal metastases are most commonly seeded from lung and breast tumours. Therefore, to address how cancer cells can thrive in the CSF, the authors developed a mouse model of leptomeningeal metastasis from metastatic breast and lung cancer cell lines. Through an iterative selection strategy, whereby parental metastatic cancer cell lines injected directly into the CSF and found to grow in the leptomeningeal space were recovered and cultured *in vitro*, a leptomeningeal metastasis derivative cell clone was generated (LeptoM). LeptoM cells could reach the leptomeningeal space and form metastases following intracardiac injection into mice. For comparison, a brain metastasis-derived cell clone (BrM) was also selected. Expression profiles of these two metastatic cell populations were revealed to be different, and C3 was identified to be specifically upregulated in the LeptoM cells.

Given that C3 was elevated in experimental leptomeningeal metastases, the authors sought to demonstrate this correlation in a clinical context. To this end, they measured C3 levels by ELISA in the CSF of 69 patients with solid tumours and clinical signs of central nervous system (CNS) metastasis. This showed that patients with leptomeningeal metastasis had the highest levels of C3 in the CSF, and that cancer cells isolated from the CSF of patients with lung and breast cancer had increased expression of C3 mRNA over that of bronchial and mammary epithelial cells. Upon depletion of C3 in LeptoM cells, metastasis to and growth in the leptomeningeal space was suppressed *in vivo*. In support of this finding, only CSF from cancer patients with leptomeningeal metastasis could sustain LeptoM cell growth *in vitro*.

The choroid plexus, a polarized epithelium, forms the blood–CSF barrier and regulates fluid flow into the CSF while preventing circulating cells from accessing the leptomeninges. Exploring how cancer cells survive in the CSF after crossing this barrier, which was shown to express the C3a receptor, C3aR, the authors used a Transwell *trans*-epithelial migration model. In contrast to the conditioned medium from parental cancer cells, that of LeptoM cells could increase the electrical conductance across a human choroid plexus epithelial cell monolayer while immunodepletion of C3 from LeptoM cell conditioned medium eliminated this capacity, indicating that C3 can disrupt barrier integrity.

Using a dot blot array to determine the profile of secreted factors in the CSF of patients with leptomeningeal metastasis before and after diagnosis, Boire *et al.* found several proteins increased upon disease progression, including amphiregulin. Addition of recombinant amphiregulin to artificial CSF was sufficient to promote the growth of LeptoM cells *in vitro*. This suggests that the reduced barrier function resulting from C3 ligation to C3aR on the choroid plexus epithelium can facilitate the transport of growth-promoting mitogens into the CSF to stimulate metastatic cancer cell growth. Lastly, pharmacological antagonism of C3aR decreased leptomeningeal metastasis from breast and lung cancer and improved survival in mice.

These data elucidate an active mechanism whereby cancer cells can breach a host epithelial barrier to colonize and adapt to the inhospitable environment of the CSF thereby avoiding host immunity and circulating chemotherapy.

Anna Dart

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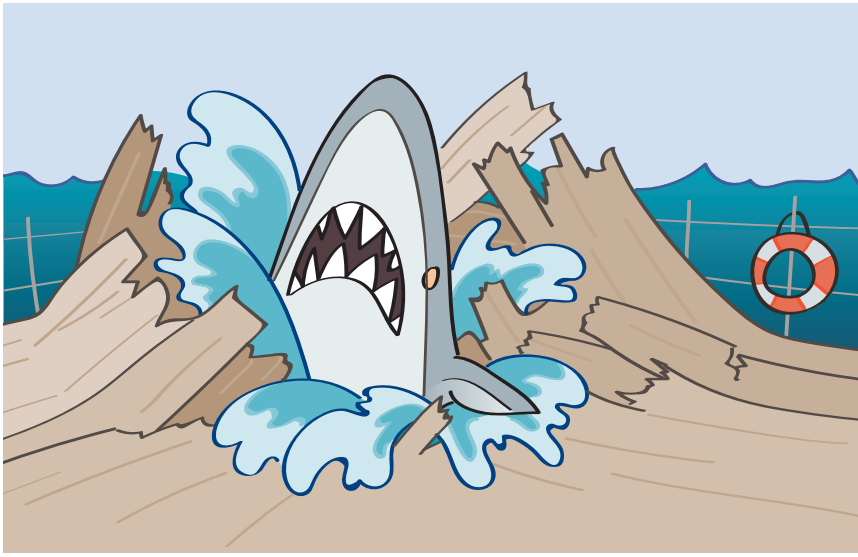

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