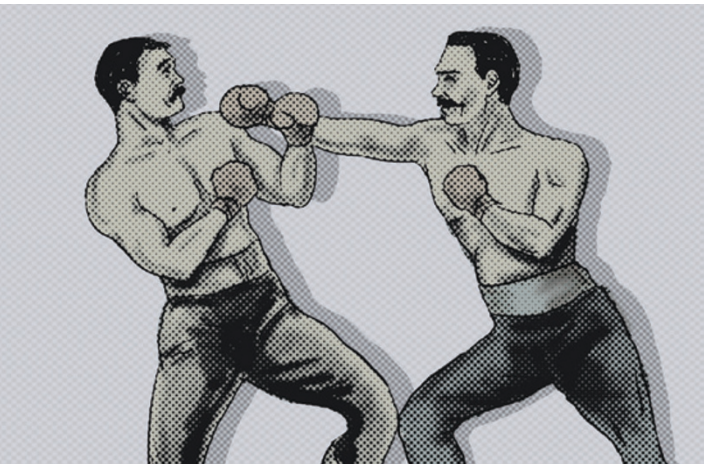
TUMOUR IMMUNOLOGY

Neutrophils fight back in the final round

The end stage of cancer progression is the dissemination of primary tumour cells to distant sites, and the formation of such metastatic tumours is the main cause of cancer-related mortality. Many studies have focused on the immunosuppressive effects of primary tumours and the mechanisms by which they can 'condition' distant tissues to be more receptive to migrating tumour cells. You could almost be forgiven for thinking that the immune system is down and out when it comes to cancer, but a new study published in *Cancer Cell* shows that neutrophils don't give in without a fight.

The authors found that the expression of several neutrophil-specific genes was increased in the lungs of mice orthotopically implanted with 4T1 mammary tumours (which spontaneously metastasize mainly to the lungs) before tumour cells could be found in the lungs. This pre-metastatic recruitment of neutrophils to the lungs was confirmed by immunohistochemical staining and was also demonstrated in a spontaneous mouse mammary tumour model.



Using an antibody specific for the neutrophil marker LY6G, the authors could deplete circulating neutrophils for up to 14 days after tumour implantation in the 4T1 model, and this covers the period during which metastatic seeding of the lungs occurs. Neutrophil depletion did not affect the growth rate or apoptosis of the primary tumour, the number of circulating tumour cells or the size of lung metastases, but it did result in a threefold increase in the number of metastatic events. Similar results were obtained from athymic mice, showing that neutrophils have a T cell-independent anti-metastatic effect that is restricted to inhibition of seeding of the pre-metastatic niche.

The formation of tumour foci in the lungs of athymic mice injected with 4T1 cells could be delayed by the transfer of neutrophils from 4T1 tumour-bearing mice but not by the transfer of granulocyte–macrophage colony-stimulating factor (GM-CSF)-induced neutrophils from tumour-free mice. In line with this, neutrophils obtained from tumour-bearing mice in several experimental models and from newly diagnosed patients with breast cancer — but not control neutrophils or neutrophils from wounded mice — were highly cytotoxic to tumour cells *in vitro*. However, there was no evidence of tissue damage despite the accumulation of large numbers of neutrophils in the pre-metastatic lung. Together, the results show that this neutrophil cytotoxicity is specifically induced by and is specific for tumour cells. The cytotoxicity was shown to require physical contact between neutrophils and tumour cells, as this contact triggered the production of hydrogen peroxide by neutrophils. Mice treated with apocynin, which inhibits the production of reactive oxygen species such as hydrogen peroxide, developed lung metastases earlier than control mice after 4T1 tumour implantation.

High levels of granulocyte colony-stimulating factor (G-CSF) were shown to be sufficient for neutrophil accumulation in the circulation and lungs, but further activation was required for cytotoxic activity towards tumour cells. Neutrophil activation, including increased hydrogen peroxide production, could be induced by the tumour-derived chemokines CCL2 and CCL5 *in vitro*. *Ccl2* knockdown in tumour cells blocked neutrophil activation (but not accumulation) at pre-metastatic sites and so led to earlier spontaneous metastasis.

The authors conclude that the activation of neutrophil cytotoxicity by tumour-derived factors such as CCL2 can contribute to the inefficiency of metastatic seeding in the lungs. Although this mechanism eventually fails in the face of a continued onslaught of circulating tumour cells, it offers the potential of therapeutic manipulation to help control metastatic disease.

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ORIGINAL RESEARCH PAPER Granot, Z. *et al.* Tumor entrained neutrophils inhibit seeding in the premetastatic lung. *Cancer Cell* **20**, 300–314 (2011)