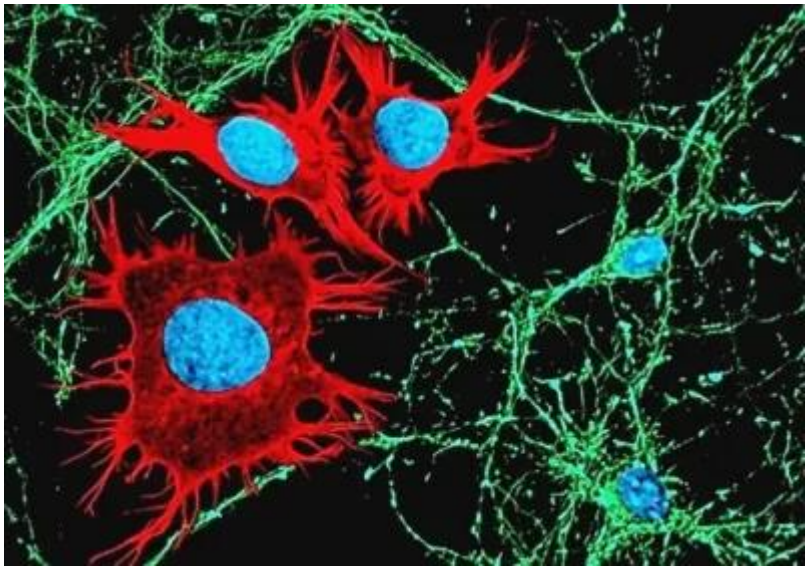
The high-grade gliomas form synapses that hijack electrical signals from healthy nerve cells to drive their growth. In this study, the authors from Germany investigated synapses between neurons and metastatic cells of non-neural cancers in the brain. They also investigated at which stage of the brain metastasis cascade synapses are formed between neurons and individual metastatic cancer cells, and whether these synaptic contacts support metastasis and cancer progression.

Glutamate AMPA receptors are heterotetrameric complexes composed of subunits GluR 1-4. These receptors mediate fast excitatory synaptic transmission in the central nervous system. The expression of Ca2+ permeable glutamate AMPA receptors in the glioma cells is a key feature of neuron-glioma synapses. The neuron-tumor synapses were not detected in brain tumors of lower malignancy (oligodendrogliomas or meningiomas). In contrast, very aggressive, incurable, primary, or secondary brain tumors receive neuronal synaptic input that drives a disease progression. It has been reported that the formation of excitatory synapses between presynaptic neurons and postsynaptic cancer cells in certain cancer types of neural origin stimulates tumor growth and invasion (Venkataramani, V. *et al. Nature 2019; 573, 532–538.* https://www.nature.com/articles/s41586-019-1564-x)



About the study

The authors assessed whether and at what stage of the brain metastatic cascade, synapses between neurons and individual metastatic cancer cells were formed.

Mice (>8 weeks old) were injected in the left ventricle with human brain metastatic cells of non-neural cancers. Female mice were used for the breast cancer model of brain metastases, and male mice for the melanoma model. To conduct intravital correlative microscopy, a chronic cranial window with a titanium ring was administered to the mice, at least three weeks before intracardial injection of human metastatic cells.

To investigate synaptic connections between neurons and brain metastatic cells, the researchers performed patch-clamp recordings of single breast cancer or melanoma cells during early growth in the perivascular niche.

Results

When circulating breast cancer and melanoma cells left the blood vessel and extravasated into the mouse brain, they were consistently found in a perivascular niche, suggesting a survival-promoting function of perivascular niche for metastatic non-neural cancer cells. Ca2+ transients were detected *in vivo d*uring metastatic seeding of the perivascular niche and early proliferation. Ca2+ activity in breast cancer micrometastases coincided with the increase in growth, which contrasted with brain metastases that were Ca2+ silent.

In 20% of single breast cancer cells or micrometastases in the perivascular niche, clear synapses were detected between metastatic cancer cells and neurons. Similarly, electron microscopy showed synapses between neurons and cancer cells in the micrometastatic stage of melanoma brain metastases.

Intravital microscopy performed in mice with metastatic breast cancer or melanoma demonstrated significantly reduced Ca2+ transients in brain cancer cells when the animals were anesthetized compared to Ca2+ transients recorded in the same brain regions when these mice were awake. Ca2+ transients recorded in cancer cells associated with neuronal activity in a crucial metastatic niche provided the first evidence of functional communication between neurons and metastatic cancer cells. The inability to induce action potentials in brain metastatic cells suggests that these cells were the receivers of unidirectional synaptic input from neurons.

Postsynaptic currents generated in synapses between neurons and brain metastatic cancer cells during their early growth in the perivascular niche were mediated by AMPA receptors, according to patch-clamp recordings. The spontaneous excitatory postsynaptic currents (sEPSCs) recorded in tumor cells confirmed that synapses between presynaptic neurons and postsynaptic tumor cells were functional. These currents demonstrated a fast rise time and exponential decay, which are the hallmarks of AMPA receptor-mediated sEPSCs. The sEPSCs in tumor cells were completely blocked by cyanquixaline, a specific antagonist of AMPA receptors. This confirmed that AMPA receptors functionally contribute to synapses between neurons and metastatic cancer cells.

To confirm the role of AMPA receptors on the growth of brain metastases *in vivo*, the scientists treated animals with perampanel, a selective and noncompetitive antagonist of AMPA receptors and an FDA-approved drug for epilepsy treatment. The administration of perampanel resulted in a lower metastatic burden and fewer brain metastases *per* mouse.

Conclusion

This study has shown, for the first time, that neurons can form physiologically relevant excitatory glutamatergic synapses with metastatic cells of non-neural cancers. This process started very early after the extravasation of cancer cells into the brain parenchyma, during their residence in the perivascular niche, which is a critical step for survival.

Cancer cells always harbored the post-synapse and never showed pre-synaptic features. Importantly, most synapses between neurons and individual metastatic cancer cells were direct synapses between presynaptic neurons and postsynaptic cancer cells, without any neuronal structure co-located on the postsynaptic side. These findings indicate *de novo* synaptogenesis in brain metastases, opposite to glioma cancer cells that showed a frequent hijacking of pre-existing brain synapses.

The excitatory postsynaptic currents generated in cancer cells were mediated by AMPA glutamate receptors, with cancer cells on the postsynaptic (receiving) side of the synapses. The discovery of Ca2+ transients, that depend on neuronal activity in breast cancer and melanoma cells, suggests a common mechanism by which synaptic interactions between neurons and cancer cells can be translated into growth-promoting signals.

The authors concluded that the discovery of synapses between neurons and metastatic cells

of tumors originating outside the nervous system was unexpected. However, it opened a new chapter in cancer neuroscience. Blocking the AMPAergic synapses between neurons and metastatic cells a selective antagonist of AMPA receptors and the approved antiepileptic drug perampanel significantly reduced the metastatic burden and opened up the possibility of a new approach to metastasis prevention.

This study has been published on a preprint server and is currently being peer-reviewed.

Journal Reference

Venkataramani V, Karreman MA, Nguyen LC, et al. Direct excitatory synapses between neurons and tumor cells drive brain metastatic seeding of breast cancer and melanoma. BioRxiv preprint. https://doi.org/10.1101/2024.01.08.574608