

## News Release

Identification of initial heterogeneous microglial responses to disseminated tumor cells

### Key Points

- **Intravital imaging captured microglia phagocytosing metastatic “seeds,” preventing colonization of the brain.**
- **Two-photon microscopy and optical tagging enable us to analyze the microglia at the tumor-immune frontline.**
- **Enhancing microglial activity in disseminated tumor stage may offer a new strategy to prevent brain metastasis.**

### Summary

A research group led by Research Fellow Takahiro TSUJI of Nagoya University Graduate School of Medicine (currently a postdoctoral fellow at the Fred Hutchinson Cancer Center, United States) and Professor Hiroaki WAKE has visualized, for the first time, the very moment when the brain’s immune cells, microglia, directly engulf and eliminate disseminated tumor cells—the “seeds” of metastasis. Using advanced two-photon microscopy, which allows unparalleled live imaging of the brain, the team captured these critical interactions in real time.

Brain metastasis remains one of the most difficult cancer complications to treat, profoundly impacting patient survival. Until now, it has been unclear why microglia, normally the brain’s sentinels, sometimes fail to prevent incoming cancer cells from growing after they arrive via the bloodstream.

In this study, the researchers recorded the “battle” between microglia and cancer cells in live mouse brains using two-photon microscopy. They observed that while some microglia actively phagocytosed and cleared tumor cells, others paradoxically supported cancer cell survival and growth. To investigate these distinct roles, the team applied their newly developed “Opto-omics” approach, in which only the microglia physically interacting with tumor cells are selectively tagged with light, isolated, and analyzed. By integrating single-cell transcriptomics with longitudinal tumor profiling, they discovered that tumor-derived molecules such as CD24 act as “don’t eat me” signals, enabling cancer cells to evade microglial attack. Removing these signals restored robust microglial phagocytosis and dramatically suppressed brain metastasis.

These findings reveal a novel therapeutic strategy: harnessing the brain's innate immune power to eliminate disseminated tumor cells before they establish metastases. The study paves the way for preventive medicine against brain metastasis.

This work was conducted in collaboration with Prof. Toyohiro HIRAI, Kyoto University, Teppei SHIMAMURA, Tokyo University of Science, and Hiroyoshi NISHIKAWA, the National Cancer Center Japan. The results were published online in *Cancer Research*, a journal of the American Association for Cancer Research on December 10, 2025 (December 11, 2025 JST).

## Research Background

Metastasis is the leading cause of cancer-related death. Among its forms, brain metastasis is particularly devastating, with limited treatment options and profound impact on both quality of life and survival. While therapies exist once sizable tumors have formed in the brain, almost no strategies directly target the very first disseminated cancer cell (“seed”) that arrives and lodges in the brain. One major reason is that these initial cells are extremely small—barely detectable even under a microscope—making their detection, isolation, and analysis extraordinarily difficult.

The brain, however, is equipped with immune sentinels known as microglia. These cells rapidly respond to pathogens and debris by engulfing and clearing them. But in the case of tumor “seeds” arriving through the bloodstream, do microglia act as defenders or as accomplices? This critical battle unfolds deep inside the brain during the first hours to days, and has long remained a black box.

Previous studies have relied largely on “snapshots” of fully developed metastases or surgically resected tissue. Such static observations could not reveal what happens in real time, or which cells directly confront cancer cells as they arrive. This limitation led to biased interpretations: the protective, phagocytic role of microglia was often underestimated, while microglia and related macrophages were more frequently classified as tumor-promoting. This prevailing view became a major obstacle in devising strategies to prevent metastasis at its earliest stage.

To overcome this barrier, the research team developed an approach to capture disseminated tumor cells in living mouse brains at single-cell resolution using two-photon microscopy. With this system, they recorded not only the moments when microglia actively eliminated tumor cells (Fig. 1), but also cases where microglia instead permitted tumor growth, enabling the formation of micrometastases. Based on these contrasting observations, the researchers hypothesized that microglia may acquire disease-specific functional states at the tumor interface.

To test this, they combined holographic two-photon microscopy with their newly established “opto-omics” platform. This allowed them to precisely tag with light, isolate, and analyze only the microglia that were physically engaging tumor cells at the frontline. By linking these dynamic, real-time interactions with downstream molecular analyses, they successfully connected tumor-microglia behavior to the underlying mechanisms at play.

This breakthrough opens the way to understanding how microglia decide between tumor clearance and tumor support, at the very first encounter. Importantly, it suggests a new therapeutic vision: amplifying the natural protective power of microglia at the earliest stage, to eliminate metastatic seeds before they take root. Such a strategy could bring us closer to a future where brain metastases are prevented before they ever begin.

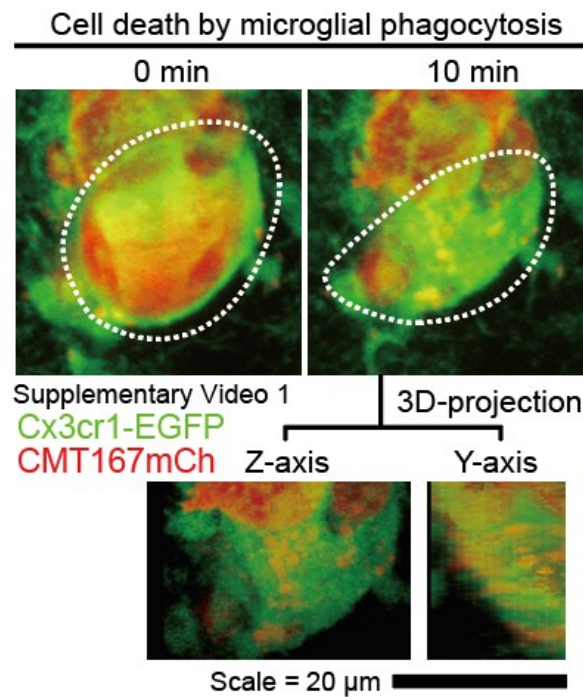


Fig. 1 Microglia can phagocytose disseminated tumor cells

## 2. Research Findings

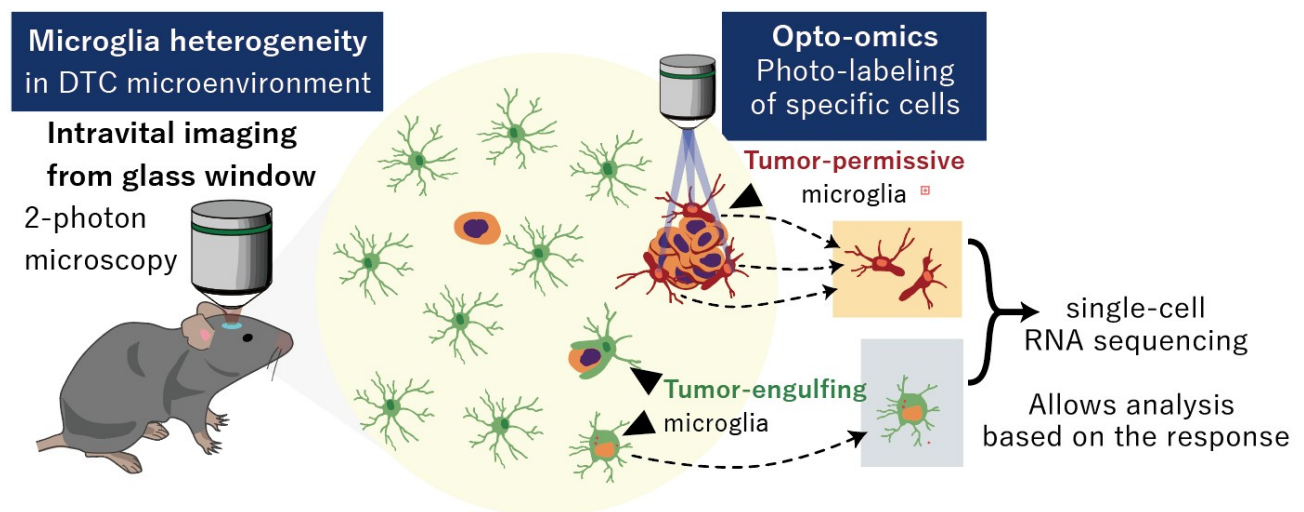


Fig. 2 Microglial heterogeneity and photo-labeling of specific microglia

### 1. Visualization of the “first moment” of brain metastasis

The research team developed a mouse model of early-stage brain metastasis from lung cancer. Using two-photon microscopy, they captured the decisive moment when microglia directly engulf and eliminate disseminated cancer “seeds.” This is the first high-resolution, in vivo recording of microglial phagocytosis of tumor cells, demonstrating that microglia can act as defenders against cancer through active engulfment (Fig. 1).

## 2. Discovery of microglial heterogeneity

Conventional understanding held that microglia primarily support tumor growth. By combining single-cell in vivo imaging with single-cell RNA sequencing, the team showed that not all microglia behave the same: some actively phagocytose cancer cells to protect the brain, while others promote tumor survival and growth. These findings provide direct evidence, from both microscopy and gene-expression patterns, that microglial responses in the brain are highly diverse (Fig. 2).

## 3. Optical tagging of tumor-adjacent microglia

The researchers further discovered that only microglia in close proximity to tumor cells undergo morphological and functional changes. To specifically analyze these frontline cells, they generated mice expressing the photoswitchable fluorescent protein **PSmOrange2**, which changes color from orange to near-infrared when exposed to a defined wavelength of light (930 nm). Using two-photon microscopy with holographic precision, they selectively illuminated only those microglia clustered around tumor cells, marking them for downstream analysis.

The photoconverted microglia were then isolated by flow cytometry and subjected to detailed transcriptomic profiling. Integrating these data with single-cell transcriptomics and computational analysis, the team identified molecular pathways involved in microglial phagocytosis of tumor cells. This approach linked direct, real-time evidence at the tumor site to underlying molecular mechanisms (Fig. 2).

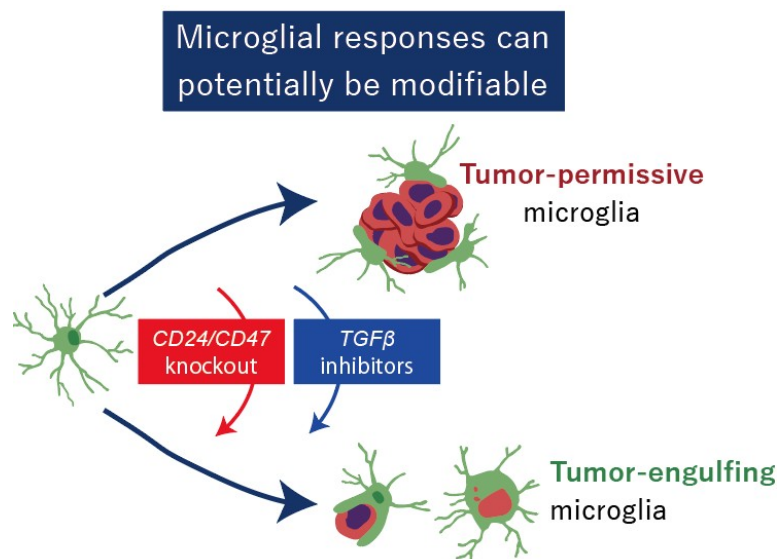


Fig. 3 Microglial responses can potentially be modifiable

#### **4. Identification of the merit of “don’t-eat-me” signals**

By analyzing tumor cells over time from dissemination to metastatic outgrowth, the researchers identified the cell-surface proteins **CD24** and **CD47** as key molecules enabling tumor cells to evade microglial attack. Blocking or removing these signals reactivated microglial phagocytosis and strongly suppressed brain metastasis (Fig. 3).

#### **5. Clues toward clinical translation**

Expression of CD24 and CD47 was also confirmed in human tumor samples, highlighting their potential as therapeutic targets and diagnostic biomarkers in brain metastasis.

### **Future Perspective**

Looking ahead, the research team aims to develop preventive therapies that maximize the power of microglia at the earliest stage—before cancer cells can take root in the brain—to “nip metastasis in the bud.” At present, molecularly targeted drugs against CD47 exist, but there are no therapies that inhibit CD24. The team will work on optimizing inhibitors and antibody drugs against these targets, as well as designing strategies that combine them with immunotherapies and existing treatments.

In parallel, they will pursue the development of predictive biomarkers to identify which patients are most likely to benefit, paving the way toward personalized medicine. Furthermore, the researchers plan to expand this framework—real-time imaging coupled with opto-omics—to other cancer types and organs, aiming to establish a versatile platform for controlling the earliest stages of metastasis. This approach may also provide new insights into the roles of microglia in other neurological diseases.

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## **Publication**

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