PRESS RELEASE

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The Wistar Institute Discovers a Promising Target in Brain Cancer

Researchers' Discovery Leads to Effective Anti-Tumor Treatment in Lab Testing

PHILADELPHIA — (February 28, 2025) — The lab of Filippo Veglia, Ph.D., at The Wistar Institute has discovered a previously unknown mechanism for how aggressive brain cancers reprogram immune system cells from fighting cancer to enabling further tumor growth. The team's findings were published in the paper "Functional reprogramming of neutrophils within the brain tumor microenvironment by hypoxia-driven histone lactylation," from *Cancer Discovery.*

Brain and nervous system tumors are some of cancer's most lethal forms; someone diagnosed with this type of cancer has a roughly one in three chance of surviving the next five years. Certain immunotherapies that stimulate the immune system to target specific cancer markers have shown progress against several brain cancers, but in many cases (and even more frequently in the most severe forms of brain cancer, like glioblastoma), the presence of tumor-infiltrating neutrophils is the key factor that has prevented these therapies from working.

Neutrophils are a type of white blood cell that the immune system uses to attack cancer in its early stages. However, scientists have discovered that, if a tumor survives the body's initial defenses and continues to grow, these tumor-associated neutrophils actually start to work for the tumor rather than against it by suppressing further anti-cancer interventions from the immune system.

Now, scientists know *how* glioblastoma reprograms tumor-infiltrating neutrophils. In their new paper, Wistar's Dr. Filippo Veglia and his team set out to understand the mechanisms behind brain cancer's reprogramming of neutrophils — and how to stop it.

Researchers investigated the subset of neutrophils found almost exclusively within the brain tumor in preclinical models of brain cancer. Analysis showed that 25-30% of these tumor-infiltrating neutrophils expressed the CD71 protein, which was notably absent from most of the other neutrophils outside the brain tumor.

The team tested the immunosuppressive activity of intra-tumor CD71 positive ($CD71^+$) neutrophils and found that they reduced immune system activity where CD71 negative ($CD71^-$) neutrophils did not. These immunosuppressive effects, the team found, were heightened in hypoxic (oxygen-deprived) environments like the hypoxic regions within the tumor where $CD71^+$ neutrophils occur. Further analysis revealed that hypoxic $CD71^+$ neutrophils expressed an additional gene, ARG1, that caused the immunosuppressive effect. Without ARG1, even hypoxic $CD71^+$ neutrophils did not suppress the immune system according to the researchers' analysis.

The hypoxic $CD71^+$ neutrophils had come to acquire ARG1 expression and its immunosuppressive effects, but researchers did not yet know how. Dr. Veglia and team suspected an interplay between hypoxia and neutrophils' glucose metabolism was the root cause; the original suspect group of neutrophils from within the brain tumor (hypoxic $CD71^+$ neutrophils) had shown increased indicators of glucose metabolism and lactate accumulation.

By inhibiting both glucose metabolism and the hypoxic $CD71^+$ neutrophils' ability to process lactate, researchers eliminated the neutrophils' ability to suppress immune responses, which proved that both glucose metabolism and lactate accumulation were critical to the immunosuppressive reprogramming.

At this point, researchers knew that hypoxic $CD71^+$ neutrophils, through glucose metabolism and lactate accumulation, acquired ARG1 expression, which would cause the neutrophils to suppress the immune system.

One crucial question remained: why would glucose metabolism and lactate accumulation cause ARG1 to be expressed?

The research team drew from an influential study that showed how gene expression could be changed through a process called histone lactylation. Histones are proteins that govern the structure of our

genes, and certain changes to histones can cause genes to be turned on or off. In histone lactylation, incompletely metabolized lactate produces by-products that attach molecules called lactyl groups to histones, and those modified histones cause changes in gene expression.

When researchers looked for signs of this histone lactylation in hypoxic $CD71^+$ neutrophils, they confirmed their suspicions. Not only did the $CD71^+$ neutrophils show higher levels of histone lactylation markers than $CD71^-$ neutrophils — the histone lactylation markers were high in the region of the ARG1 gene, an indication that the histone lactylation process had caused the ARG1 gene to be turned on. By selectively turning off the neutrophils' ability to carry out histone lactylation, the researchers successfully reduced ARG1 expression.

Dr. Veglia and team discovered the central process causing neutrophil reprogramming: neutrophils infiltrate the brain tumor; hypoxic tumor regions recruit neutrophils, including those expressing CD71; the hypoxic $CD71^+$ neutrophils increase their glucose metabolism, which causes lactate production to increase; the excess lactate causes histone lactylation; the histone lactylation causes ARG1 expression; and the ARG1 expression suppresses the activity and signaling of other immune cells.

Using their knowledge of the neutrophil reprogramming process, the team developed a therapeutic approach to stop the pro-cancer effect. They used the anti-epileptic compound isosafrole, which inhibited a key lactate-processing enzyme. In preclinical laboratory testing, isosafrole treatment reduced histone lactylation, resulting in an impaired ARG1 expression and immunosuppression of hypoxic $CD71^+$ neutrophils, without negatively affecting other immune cells. By combining isosafrole treatment with a targeted brain cancer immunotherapy — which has previously struggled to succeed due to the cancer's immunosuppression — Dr. Veglia and team overcame the resistance to immunotherapy and substantially slowed tumor progression in preclinical models.

"Our work shows the step-by-step process of how brain tumors can cause an immune system's neutrophils to become deadly barriers to cancer treatment," said Dr. Veglia. "Now that we understand this reprogramming process, we know how to interrupt it, and already, preclinical data show that isosafrole treatment that disrupts neutrophil reprogramming can make poor-prognosis brain tumors responsive to immunotherapy. We look forward to seeing how future research can refine this strategy to fight some of the deadliest cancers."

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Work supported by: This work was supported by The Ben & Catherine Ivy Foundation Emerging Adult Glioma Award; National Institutes of Health grants R01 NS131912, P30 CA076292, and P30 CA010815; PRIN 2022 grant 2022M5LBKP; and Sapienza University of Rome grant RM1221816BCE0EAA.

Publication information: "Functional reprogramming of neutrophils within the brain tumor microenvironment by hypoxia-driven histone lactylation," from *Cancer Discovery.*

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