'Dead zone' within tumor promotes cancer spread, helped by a protein secreted by cancer cells

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Fred Hutch's Ami Yamamoto, foreground, at work in the lab of Dr. Kevin Cheung (seen in background). Yamamoto's investigation of the necrotic core of breast cancer tumors led to an important discovery as to how cancer metastasizes. Credit: Robert Hood / Fred Hutch News Service

A tumor's necrotic core contains factors that appear to promote metastasis, or the seeding of tumors cells throughout the body, according to a new study in rats by researchers at Fred Hutchinson Cancer Center.

Researchers hope their findings, which will be published the week of Feb. 27 in the *Proceedings of the National Academy of Sciences*, lead to a better understanding of how to cure metastatic, or stage 4 cancers, which are treatable but not curable.

"Tumor necrotic cores are a fairly common phenomenon, but they haven't been linked to cancer metastasis until recently," said lead author Ami Yamamoto, a Fred Hutch graduate student working in the Cheung Lab. "Our research put together observations other people have made into the specific context of breast cancer metastasis. Our work shows a link between necrosis, circulating tumor cells and cancer metastasis."

What is a dead zone

Necrotic cores are tumors that are dying from the inside out, and they make for a perfect environment for cancer to spread.

"Dead zones of tumors have leaky blood vessels, hypoxia or low levels of oxygen and the recruitment of immune cells, some of which have been shown to help cancer cells spread," Yamamoto said. "What we think is happening is that the necrotic core is mostly a dead zone, but it also has some surviving tumor cells that help the cancer disseminate in the body."

Surgeons, pathologists, radiologists, clinicians and researchers often come across necrotic cores in their line of work and they're usually not a good sign.

"Necrosis is a clinical finding seen in aggressive tumors that grow quickly," said Fred Hutch physician-researcher Kevin Cheung, MD,

senior author on the study. "When we see it in a patient's biopsy, it means this is a dangerous tumor that needs to be treated aggressively."

But necrosis isn't only something seen in large, late-stage tumors, Cheung said. It can happen in early-stage and small tumors too.

A protein linked to necrosis and cancer spread

The Fred Hutch team developed a new rat model of breast cancer metastasis to study the necrotic core of tumors. Over several weeks, Yamamoto tracked the progression of circulating tumor cells (CTCs), which is a measure of whether the cancer cells are escaping into the bloodstream to spread throughout the body. They found zero CTCs at the first two time points examined (after 13 and 17 days), but that changed by the fourth time point at 27 days.

"Suddenly, we found hundreds of CTCs," Yamamoto said. She linked the increase in cancer cells with when the primary tumor developed a large central area of necrosis.

Further investigation showed a stark difference in gene expression between the necrotic and the non-necrotic regions of the tumor.

"We found that a gene which encodes angiopoietin-like 7, a secreted protein, was the most enriched tumor-derived gene in the necrotic and regions next to necrotic regions of the tumor," Yamamoto said.

The researchers found that this single protein, angiopoietin-like-7, remodels the tumor microenvironment, somehow encouraging the tumor cells to grow past their nutrient limits, undergo necrosis and start spreading to other parts of the body.

"This was a surprise," Cheung said. "We thought necrosis was entirely

unregulated, not something you can control."

Yamamoto then did experiments to see how controlling the protein would impact necrosis.

"When we suppressed the expression of this protein in the tumors, there was a dramatic reduction of necrotic tumor area," Yamamoto said. "Suppression of angiopoietin-like 7, or A-7, also reduced circulating tumor cells to almost zero and reduced distant metastases and dilated, large blood vessels."

Yamamoto said their research not only showed A-7 regulates the development of central necrosis in the primary tumor, but also the development of dilated blood vessels which could be helping the dissemination of circulating tumor cells and metastasis.

A potential new target for treatment

Beyond the surprise of such an important mechanism to necrosis, these findings unveiled the potential for a new targeted treatment for patients.

"Our ultimate goal is to develop a therapeutic antibody against A-7 that will prevent or reduce metastasis in patients with metastatic breast cancer," she said.

Toward that end, the team has already come up with over 200 candidates for an anti-A-7 antibody but need to screen through all of them to see which ones do the best job of blocking the function. Cheung said he and his team also want to analyze the data of large patient cohorts to better understand the role between necrosis in the tumor and the risk of metastatic dissemination.

"Some patients have evidence of markers of necrosis in their blood," he

said. "That suggests this is potentially happening not just in preclinical models but in patients."

Cheung and Yamamoto also want to delve into the unanswered questions that remain.

"Before we started this, we probably considered the necrotic core to be the least interesting part of the tumor," Cheung said. "We're now confident the inside is a really interesting place for studying the source of metastatic cancer. There's more cancer biology there that awaits."

More information: Yamamoto, Ami et al, Metastasis from the tumor interior and necrotic core formation are regulated by breast cancer-derived angiopoietin-like 7, *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2214888120. doi.org/10.1073/pnas.2214888120

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