Cracks in the armor of therapy-resistant cancer cells

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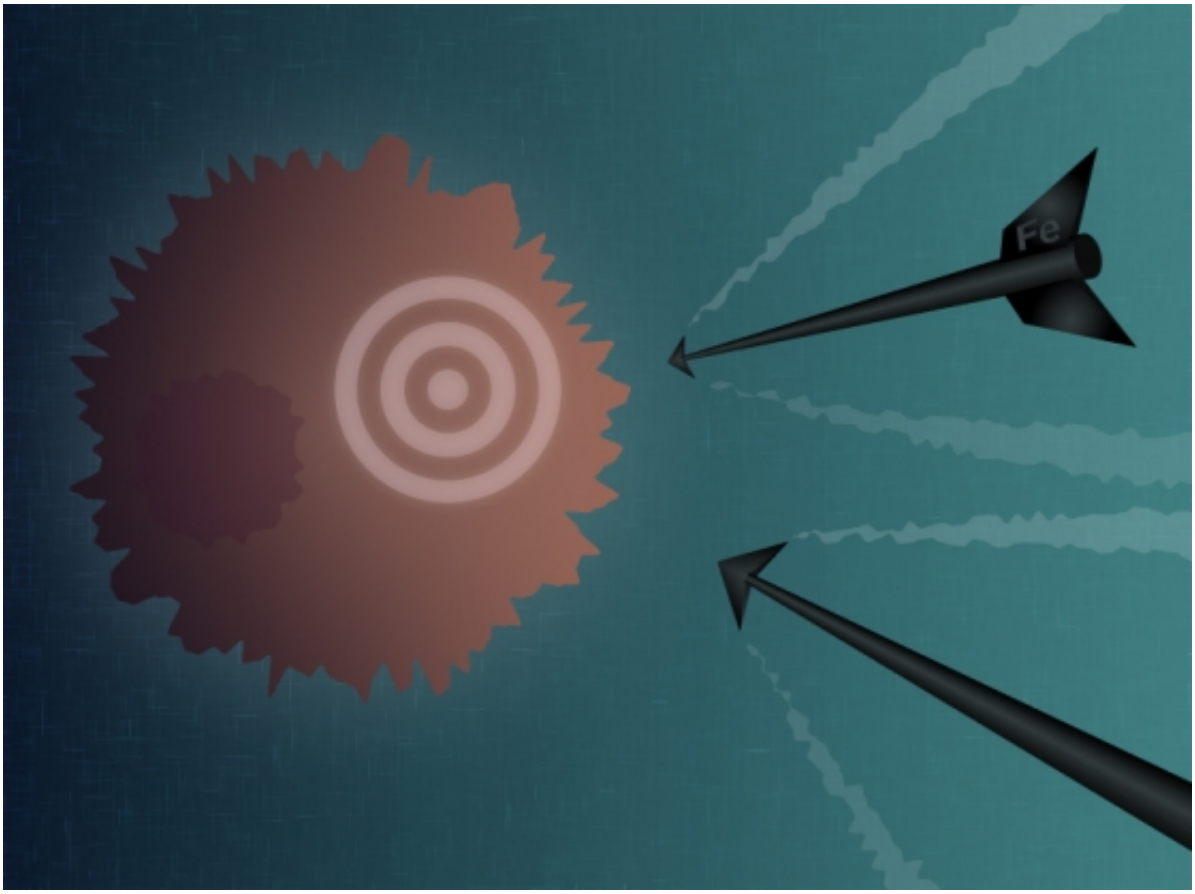

Credit : Susanna M. Hamilton, Broad Communications

A new study shows that cancer cells across multiple lineages can adopt the same therapy-resistant cell state, enabling broad resistance to targeted therapies. However, the resulting cell circuitry also results in a

vulnerability that can be exploited to induce a form of cell death called ferroptosis.

Despite a clinician's best efforts with the current arsenal of cancer treatments, tumor cells have revealed a powerful ability to resist therapeutic strategies, escaping treatment and flying under the radar until the patient suffers a recurrence. Impressive and seemingly successful responses to therapy are often only temporary.

To develop more enduring therapeutic approaches that can eradicate cancer, researchers and clinicians have been hunting for defining features that enable tumor cells to resist therapies. Resistance is commonly viewed by researchers and clinicians as a function of genetics: Mutations that prevent a drug from binding its target, or that allow the cell to utilize alternate molecular pathways, arise during prolonged therapy exposure. Proposed solutions to mutation-based resistance tackle the problem on a mutation-by-mutation, or drug-by-drug, basis.

But another approach also considers resistance a result of cell plasticity, where cancer cells adopt new, resistant states initially through alterations in transcriptional programs, without the use of genetic mutations—which may arise later and stabilize the resistance.

Now, a team led by researchers from the Broad Institute of MIT and Harvard has determined that such cell state changes play a dominant role in the ability of many types of cancers to initially resist therapeutic attacks. The investigation was published today in *Nature*.

Previous work has demonstrated that many cancer cells are able to transition to one or more quasi-stable cell states with mesenchymal characteristics. A key feature of these mesenchymal states appears to be a reduced capacity to undergo apoptotic cell death, a normal process of

programmed cell death that many cancer therapies try to jumpstart in tumor cells. In that light, the researchers hypothesized that a mesenchymal cell state may form the basis of a common therapy-resistant state in cancer cells. However, the molecular underpinnings of this mesenchymal state, and how similar it may be across different cancer lineages, remained unknown.

The new investigation demonstrates that, in multiple types of cancer cells, a therapy-induced change to a certain type of mesenchymal cell state confers broad resistance to targeted therapies. Illuminating the molecular characteristics of this state revealed a vulnerability that enables the resistant cells to be eliminated through a form of cell death called ferroptosis. The work suggests a radically new way to combat resistance—one that focuses on an epigenetic cell state, common across cancer lineages, rather than individual genetic mutations that follow.

Understanding resistance

In order to understand the transition of a cancer cell to a mesenchymal cell state and its association with resistance, the researchers first had to determine how commonly it occurred. Across a panel of 473 epithelium-derived cancer cell lines in the Broad-Novartis Cancer Cell Line Encyclopedia, the team searched for gene expression signatures that were previously correlated with a mesenchymal cell state predicted to yield resistance in human melanomas. "We used those signatures as an anchor to look through these data," said Vasanthi Viswanathan, a postdoctoral fellow at the Broad Institute and first author on the study.

Once the researchers had established a mesenchymal "score" for each cell line, representing this particular mesenchymal state, they examined how that score might correlate with resistance to targeted therapies. The team drew on data from small-molecule screens described in the Cancer Therapeutics Response Portal, a database developed by researchers at

Broad and sponsored in part by the National Cancer Institute. The wealth of screening data allowed the researchers to model the effect of targeted therapies on the cancer cells and determine whether any signs of resistance were associated with a high mesenchymal score.

Indeed, the higher mesenchymal scores correlated with resistance to targeted therapies in epithelium-derived cancer cell lines, which aligned with the earlier data from human patients. Notably, the mesenchymal signatures also correlated with suppression of apoptosis and resistance to therapy-related compounds in other cancer cell lineages. The cell state shared features with other mesenchymal states described in previous literature, but didn't line up perfectly with them, explained Viswanathan—this was something novel.

"We suspect that this cell state might be an evolutionarily conserved stress response that comes into play when a cancer cell is exposed to an apoptosis-inducing treatment," said senior author Stuart Schreiber, a core institute member and co-founder of the Broad Institute, professor at Harvard University, and Howard Hughes Medical Investigator. "With their intrinsic cellular plasticity, a few cells seem to epigenetically switch their state to one without the ability to undergo apoptotic death. They just hunker down and survive. It appears to be a common response of many, if not all, cancers to whatever therapeutic strategy you use."

But this survival strategy isn't bulletproof.

Cracks in the armor

When the team examined the data for small molecules that did have an effect on these resistant cells, the researchers found that gene expression associated with the mesenchymal cell state correlated with vulnerability to compounds known to induce ferroptosis—a form of cell death, distinct from apoptosis, in which iron catalyzes the formation of toxins

in the cell. Ferroptosis has been described in the scientific literature only over the last decade or so.

These ferroptosis-inducing compounds effectively targeted tumor cells from multiple lineages that had transitioned into the characterized mesenchymal cell state, as well as cells from cancers that have a mesenchymal origin and ovarian and kidney cancers (which grow from organs generated by the mesenchymal layer in embryos).

The most effective ferroptosis-inducing compounds attacking these cells were ones that interfered with function of the gene GPX4. Cells from many different cancers, including non-small cell lung cancer cells, pancreatic cancers, prostate cancers, and melanomas, were sensitive to GPX4 inhibition after acquiring this mesenchymal state and resistance to various targeted therapies. The sensitivity also held in vivo when tested in mice.

Mapping the vulnerability

The researchers dove in to investigate the connection between this therapy-resistant mesenchymal cell state and GPX4, and ultimately defined a pathway underlying the dependency on GPX4 expression.

"This cell-state change is all about lipid biochemistry," explained Schreiber. Normally, GPX4 functions in a pathway used to create lipid messenger molecules. This pathway centers on the synthesis, storage and use of long-chain polyunsaturated fatty acids, which are susceptible to becoming reactive lipid peroxides. During the process, GPX4 breaks up these lipid peroxides, removing the toxic intermediates.

The therapy-resistant mesenchymal cancer cells utilize this pathway more than healthy cells typically do, according to the team. "If these lipid hydroperoxides don't get quenched, they react with iron and lead to

ferroptosis—so GPX4 sits there and cleans them up," said Viswanathan. "And in these therapy-resistant cells, if you inhibit GPX4, the lipid hydroperoxides build up and ferroptosis occurs."

Viswanathan, Schreiber, and their colleagues also uncovered another player: the gene ZEB1. When the researchers knocked out this gene, the cancer cells lost their sensitivity to GPX4, indicating that ZEB1 provides a bridge between mesenchymal gene expression and lipid-peroxide vulnerability. Growing evidence has shown that ZEB1 directs the uptake, accumulation, and mobilization of lipids, and affects mesenchymal-associated remodeling of the plasma membrane.

The team is continuing to validate their observations and investigate the other mechanistic changes that occur when cancer cells convert to this therapy-resistant mesenchymal state. The study results reveal a potential target for the development of new therapies aimed at enhancing cancer treatments and combating resistance.

"We've highlighted a profile of therapy-resistant cells that appears common and conserved across multiple types of cancer," explained Viswanathan. "These mesenchymal GPX4-dependent cells appear to represent a version of the cancer that's almost 'forgotten' its originating tissue. It may allow cells to escape therapies designed to treat a specific kind of tumor because they no longer resemble that kind of tumor. But with this universality, we've been able to figure out a way that we might be able to kill them."

More information: Vasanthi S. Viswanathan et al. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway, *Nature* (2017). DOI: 10.1038/nature23007

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