DNA deletions promote cancer, collateral damage makes it vulnerable

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Genomic deletions promote cancer by carving up or eliminating tumor-suppressor genes, but now scientists report in the journal *Nature* that the collateral damage they inflict on neighboring genes exposes cancer cells to vulnerabilities and new avenues for attack.

Working with cell lines of glioblastoma multiforme, the most lethal type of brain tumor, researchers from the Dana-Farber Cancer Institute at Harvard Medical School, and some now at The University of Texas MD Anderson Cancer Center, found that collateral deletion of a gene vital to tumor metabolism allowed them to kill malignant cells by blocking another gene that redundantly performs the same function.

"Cancer-driving deletions disable tumor suppressors, and so far efforts to restore or replace the function of these deactivated genes, or turn them against cancer cells, have yet to show promising results," said co-lead author Florian Muller, Ph.D., an instructor in MD Anderson's Department of Genomic Medicine.

Passengers matter - a new approach for targeted therapy

"In this case, we looked at passenger deletions - genes co-deleted along with tumor-suppressor genes, but not directly involved in cancer promotion - as a starting point for identifying potential targets and therapies," Muller said.

The researchers wiped out glioblastoma cells that had deletions of the metabolic gene ENO1 on both copies of chromosome one when they also inhibited the function of ENO2, which is located on chromosome seven. Both genes encode for the enzyme enolase, which carries out a crucial step in glycolysis, the processing of glucose into energy that is particularly important to solid tumors. Cells can tolerate the loss of either ENO1 or ENO2, but not both.

"The principle of collateral vulnerability caused by passenger deletions of redundant essential genes provides the basis for a new approach to identify potential targets and develop targeted therapies," said MD Anderson President Ronald DePinho, M.D., senior author of the paper.

"These deletions are found in hundreds of genes in many types of cancer, so our model for glioblastoma multiforme should apply to developing personalized treatments for other cancers as well," DePinho said.

Targeting gene loss of function in tumor cells stands in contrast to the more frequent search for molecular targeted therapies to stymie active cancer-driving genes that are amplified or dysfunctional due to mutations.

Finding ENO

DNA deletions that take out tumor-suppressing genes tend to be large events that affect many other neighboring genes. "Most metabolic genes come in duplicates; they back one another up," Muller said. The hypothesis: Deletions create vulnerabilities by deactivating both copies of one essential gene, providing an opportunity to treat cancer by deactivating the second gene

A search of The Cancer Genome Atlas for glioblastoma multiforme

yielded a variety of candidates, including ENO1, which resides on a stretch of chromosome one that includes several candidate tumor-suppressing genes. That portion of the chromosome is deleted in 1 to 5 percent of glioblastomas and in scattered cases of other tumor types.

In mammals, the enzyme enolase is encoded by ENO1 in all types of tissues, by ENO2 in neural cells, and by ENO3 in muscle cells. In theory, with ENO1 missing, inhibiting ENO2 would thwart glioblastoma cells without harming normal brain cells, which would still have both genes.

shRNA knock down of ENO2 confirms hypothesis

In work conducted by co-first authors Simona Colla, Ph.D., also an instructor in Genomic Medicine, and Elisa Aquilanti, a medical student at Albert Einstein College of Medicine in New York, the team used short hairpin RNA to knock down ENO2 in glioblastoma cell lines either missing ENO1 or with ENO1 intact.

Knocking down ENO2:

- Had no effect in glioblastoma cells with ENO1 intact.
- Sharply inhibited growth of glioblastoma cells with ENO1 deleted and caused complete loss of tumor-forming potential when injected into the brains of mice.

As expected, growth could be restored in ENO1-deleted glioblastoma cells by either expressing hairpin-resistant ENO2 or by artificially expressing ENO1 in the cells.

"In cells with ENO1 deletions, the backup is gone. We then hit ENO2 with a gene-specific shRNA, no enolase is left and the cells cannot survive," Muller said.

An enolase inhibitor is selectively toxic to ENO1-deficient cancer cells

Because ENO1 accounts for 75 to 90 percent of total enolase activity, ENO1-deleted glioblastomas cells have much lower overall enolase activity than either cancer cells with ENO1 intact or normal non-cancerous cells. With ENO1-deleted cells already deficient in the enzyme, the investigators reckoned that low doses of a small-molecule enolase inhibitor would be sufficient to block glycolysis and reach a toxic threshold.

The enolase inhibitor phosphonoacetohydroxamate (PHAH) was found to be highly toxic to ENO1-deleted cancer cells while having minimal effect on ENO1-intact cancer cells or normal human brain cells. This toxicity could be completely reversed by artificial re-expression of ENO1.

PHAH isn't approved for human use, Muller said, and based on its structure is unlikely to penetrate tissues and tumors effectively. Researchers are working with MD Anderson's Institute for Applied Cancer Science and others to develop a potential drug.

Concept applicable to other passenger deletions, other cancers

Although ENO1 deletions only occur in a small subset of glioblastoma patients, passenger deletions are quite frequent in the cancer genome and occur in most cancer types, the investigators noted. Because genes critical for cell survival often come in ENO1/ENO2 type duplicate pairs, the concept of collateral lethality is likely to be applicable to passenger deletions beyond those affecting ENO1.