Study shows how some intestinal cells resist chemotherapy and radiation

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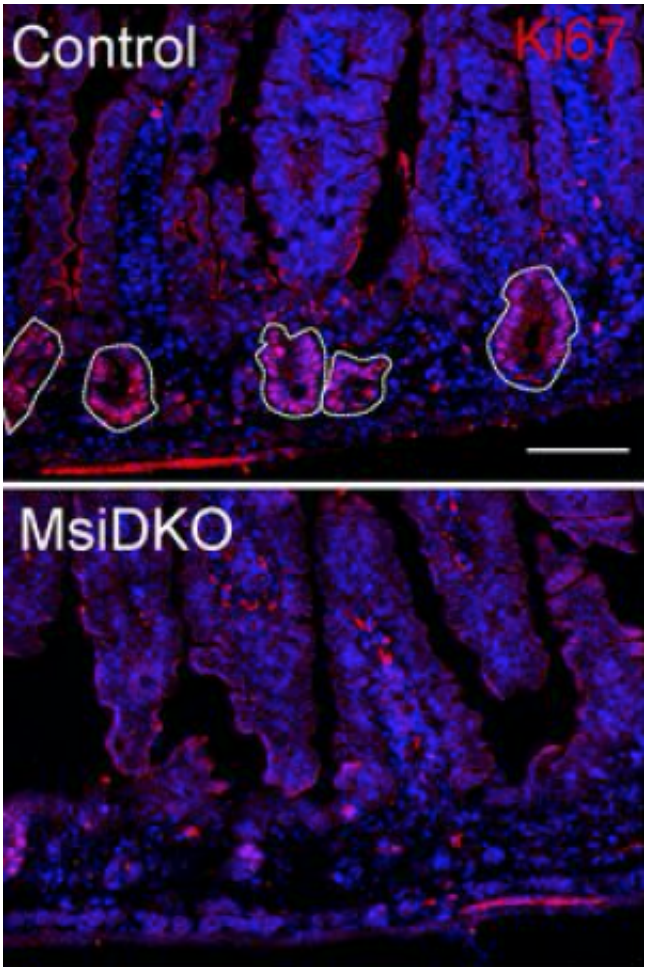

After radiation, normal mice began to regrow their intestinal epithelial tissue (top), but those lacking MSI (bottom) couldn't. Credit: University of Pennsylvania

When treating cancer with chemotherapy and radiation, decisions about dose must walk a fine line between attacking cancerous cells and preserving healthy ones. Overly aggressive radiation therapy to the torso, for example, can damage the epithelial cells that line the intestines, leading to chronic gastrointestinal problems.

Yet some intestinal cells can withstand chemotherapy and radiation. New insights from a University of Pennsylvania-led team reveal how these cells remain protected from damage, serving as an injury-resistant pool from which the intestinal epithelium may regenerate. These "reserve" stem cells, the team found, are protected because they are in a dormant state, but can be pushed out of dormancy and into the cell cycle by RNA-binding proteins in the Musashi family.

Interestingly, earlier work by the Penn researchers found a role for Musashi proteins in driving colon cancer and that genetically inhibiting them could prevent mice from getting cancer.

"We knew that these Musashi proteins were important oncogenes and that if we inhibited them we could inhibit cancer, but now we see the downside," said Chris J. Lengner, assistant professor in School of Veterinary Medicine's Department of Biomedical Sciences at Penn and senior author on the study. "The downside is, when you delete them, the reserve stem cells can't become activated and tissue cannot regenerate in the face of injury."

The findings point to a strategy whereby intestinal tissues could be shielded from damage prior to radiation, thus promoting tissue regeneration once the treatment is completed. But they also hint at a way in which a theoretical cancerous reserve stem cell might "hide out" in the tissues in a quiescent state and avoid being killed by radiation or chemotherapy.

"We see this common theme emerge over and over," Lengner added, "which is that tumor suppression comes at the cost of regenerative capacity."

The study appears this week in the *Journal of Cell Biology*. In addition to Lengner, the study was coauthored by first author Maryam Yousefi, Ning Li, Angela Nakauka-Ddamba, Shan Wang, Kimberly Parada and Jenna Schoenberger; Zhengquan Yu of China Agricultural University; Shane T. Jensen of Penn's Wharton School and Michael G. Kharas of Memorial Sloan-Kettering Cancer Center.

Last year, Lengner's lab group showed that the Musashi proteins MSI1 and MSI2 were crucial drivers of colon cancer. Functionally redundant, they bind to RNA transcripts of genes that can help cancer achieve its rapid growth.

The group found that, when both MSI1 and MSI2 were deleted in mice, the animals appeared to be healthy and, moreover, were resistant to cancer. On the surface, this made the Musashi proteins enticing targets for a cancer treatment.

In the new study, the researchers took a closer look at how the MSI proteins functioned in mice. Again using at mice with both MSI genes deleted, they examined in detail the effects of the deletion on the intestines in general and in particular on highly proliferative stem cells in the intestine called the crypt base columnar cells. Similar to their previous work, the team found no obvious effects and no impact on the ability of the CBCs to proliferate.

The researchers had previously implicated the Musashi proteins in promoting colon cancer through a molecular pathway that is required to regenerate tissues after injury. To determine whether MSI might be involved in this regenerative ability, they examined the intestines of mice

lacking MSI1 and MSI2 that were also subjected to radiation injury, finding intestinal regeneration severely compromised compared to mice with intact Musashi proteins.

A growing body of research suggests that reserve stem cells, which make up just a fraction of one percent of total intestinal epithelial cells, are radiation- and chemotherapy-resistant and thus largely responsible for regenerating intestinal tissue after, for example, cancer treatment.

Indeed, when the researchers knocked out Musashi genes in only this type of cell, then exposed mice to radiation, they observed the same effect they had seen when they knocked the genes out in all of the intestinal epithelial cells: very poor intestinal epithelial tissue regrowth.

"The fact that the loss of these genes in these very rare cells gives you the same phenotype as the loss of these genes through the whole epithelium speaks to the importance of these reserve stem cells in the regenerative process," said Lengner.

Further experiments showed that Musashi genes were required for the reserve stem cells to proliferate, that this cell population is essentially dormant, or quiescent, and that MSI genes were necessary for the cells to move from the quiescent state into the cell cycle to grow and replicate themselves.

"When we delete these genes in the actively cycling stem cells, there is no observable effect," said Yousefi. "However when we delete these genes in the reserve stem cell compartment, we see that these cells can't leave quiescence and enter the cell cycle and also they cannot produce progeny under basal conditions. And in the case of injury they cannot regenerate the epithelium."

This quiescent state is a protective one for the cells, the researchers

noted.

"The reason that these so-called reserve cells are able to resist the radiation, we think, is because they are in this dormant state, they are not cycling and there is some evidence that in this state the DNA is very compact and thus physically resistant to damage," said Lengner.

In a final experiment to confirm the importance of both MSI proteins and reserve stem cells in regeneration after injury, the research team gave a "pulse" of MSI to reserve stem cells to kick them into the cell cycle, then applied radiation. Because MSI coaxed the once-dormant reserve stem cells to grow again, they became vulnerable to radiation and, as anticipated, the intestinal epithelium of the animals was greatly compromised.

Reversing such an approach, encouraging more cells to remain quiescent, could be one way of protecting intestinal tissue from damage during radiation treatment.

"If you were to protect the cells and keep them in a more dormant state at the time of radiation," Lengner said, "you would expect the patient to avoid some of those nasty gastrointestinal complications of the treatment."

Yet this dormancy might also work against cancer therapy. Some researchers believe that some cancers may originate from a "cancer stem cell" that quietly lurks in tissues and causes malignancies to reemerge long after an otherwise apparently successful treatment. It's possible this activation could be governed in part by Musashi proteins.

In follow-up work, the researchers hope to test whether MSI proteins are active in response to not just radiation damage but other forms of injury. They would also like to identify cell-surface markers that distinguish the

reserve stem cells from other cell types to make it easier to study this cell type in humans.

More information: Maryam Yousefi et al, Msi RNA-binding proteins control reserve intestinal stem cell quiescence, *The Journal of Cell Biology* (2016). DOI: 10.1083/jcb.201604119

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