Mutant Kras drives pancreatic cancer maintenance via metabolic pathways

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A genetic mutation that drives the initiation of pancreatic cancer also manipulates metabolic pathways to support tumor growth and progression, scientists report in the journal *Cell*.

This newly discovered role for the Kras oncogene opens up a new category of potential targets for thwarting the influential mutation, which has proved difficult to attack directly, said study co-lead author Haoqiang Ying, Ph.D., instructor in The University of Texas MD Anderson Cancer Center Department of Genomic Medicine.

Pancreatic ductal adenocarcinoma has a five-year survival rate of about 5 percent. The National Cancer Institute estimates 43,920 new cases will be diagnosed in 2012 and 37,390 patients will die of the disease.

"Our research provides an additional metabolism angle to the search for therapeutic targets," Ying said. "The highly coordinated regulation of anabolic glucose metabolism by the Kras oncogene also indicates the need to develop combination strategies to target those pathways as well as other functional components of Kras signaling."

The Kras gene produces a protein that's influential in normal cell signaling. Mutated versions of Kras get stuck in a state of permanent activation and are so tightly bound to the enzyme GTP that small-molecule inhibitors have so far been unable to successfully target it, Ying said.

Cancer initiator also fuels growth and progression

"Activating mutations in the Kras oncogene are nearly universal in pancreatic cancers and have been shown to be important in the initiation of these cancers," said study senior author Ronald DePinho, M.D., president of MD Anderson. "The role of this critical gene in the maintenance of these tumors in an in vivo setting had not been explored."

To address this question, DePinho's team at Harvard Medical School and Dana-Farber Cancer Institute, where this research originated, developed an inducible Kras transgene (iKras) and bred it into mice. This allowed the scientists to initiate expression of Kras in the pancreas by treating the mice with doxycycline and turn it off by withdrawing the drug.

Ying was a postdoctoral fellow in DePinho's lab at Dana-Farber before moving to MD Anderson after DePinho became president last September.

"These studies not only established that oncogenic Kras serves a tumor maintenance role, they allowed us to explore exactly how this occurs by extinguishing Kras in fully formed tumors," DePinho said.

Kras extinction causes tumor regression, deterioration

Pancreatic cancer progression is accompanied by the inactivation of a variety of tumor-suppressing genes. The team crossed its inducible Kras mice with others that had the tumor-suppressor p53 knocked out. Mice with either only iKras or iKras and deficient p53 were treated with doxycycline at three weeks of age.

- All iKras mice with p53 deficiency died of pancreatic cancer between 11 and 25 weeks of age with median survival of 15 weeks.
- The iKras/p53 mutant tumors had features commonly found in human pancreatic cancer, such as glandular tumor structures, invasion of local organs and metastases to the lung and liver.

Withdrawing doxycycline renders the iKras transgene extinct within 24 hours. Mice with iKras without p53 developed invasive pancreatic cancer by week eight after treatment.

When the researchers withdrew doxycycline at week nine:

- Tumors swiftly regressed in size and visibly deteriorated after 48 hours.
- After a week, MRI showed a reduction in tumor mass of about 50 percent, and PET/CT scans showed cessation of glucose uptake by the tumors.
- Programmed cell death increased, tumor cell proliferation dropped and supportive tissue in the tumor - stroma - also was altered.
- Signaling by downstream components of the Kras pathway, such as MAPK and mTOR, dropped.

Kras supports production of building blocks for cancer growth

Transcriptome analysis of gene activation in the iKras/p53 null tumors after Kras extinction indicated decreased activity of genes involved in metabolism.

Metabolic studies showed that Kras extinction led to decreases in intermediates involved in glucose metabolism and reduced glucose

uptake and lactate production. The metabolic changes correlated with the gene expression changes identified by transcription analysis.

The affected glucose intermediates are used in other glucose-utilizing pathways, which led the team to analyze the hexosamine biosynthesis pathway (HBP) and the pentose phosphate pathway, both of which are involved in the construction of cellular building blocks, including assembly of DNA and RNA.

They found that with Kras extinct, protein glycosylation via the HBP, an important signaling step in many cellular processes, is reduced to levels that are observed under conditions of glucose starvation.

The pentose phosphate pathway (PPP) uses glucose to generate ribose rings, an essential component of DNA and RNA, and to maintain the cell's ability to store electrons in NADPH. This pathway has two arms, a well-characterized oxidative arm and a less well-understood nonoxidative arm.

Kras extinction had no effect on glucose flux through the oxidative arm but led to decreases in glucose levels in components of the nonoxidative arm involved in ribose synthesis. Radioactive labeling of C-glucose showed a dramatic drop in labeled glucose incorporated into DNA/RNA when Kras is extinct.

Expression levels of enzymes involved in the nonoxidative PPP arm also fell under Kras extinction. Knockdown of either Rpia, Rpe or both enzymes reduced glucose flux into DNA/RNA and also suppressed tumor growth.

Inhibiting MAPK, Myc is similar to Kras extinction

The team then inhibited separate components of the Kras pathway to

identify potential molecular culprits in the reprogramming of the metabolic pathways to support pancreatic cancer. They found that MAPK inhibition partially recapitulated the effect of Kras extinction on glycolysis and HBP and PPP.

Knockdown of the Myc protein in iKras pancreatic cancer cells also reduced the expression of metabolism genes in glycolysis, HBP and PPP.

"We're working with MD Anderson's Institute for Applied Cancer Science to identify and validate metabolism gene targets for drug development," Ying said. The inducible Kras mouse model will be used to characterize the therapeutic potential for candidate drugs.

Provided by University of Texas M. D. Anderson Cancer Center