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Researchers Identify Distinctive Signature for Metastatic Prostate Cancer

Howard Hughes Medical Institute researchers have identified a telltale change in cellular machinery that could help clinicians predict whether prostate cancers are likely to spread or remain relatively harmless in the prostate.

The researchers found that a cellular signaling molecule called Hedgehog, which drives normal development and regeneration of prostate tissue, is greatly activated in prostate cancers. This elevated activity distinguishes dangerous metastatic cancers - those that are likely to spread - from those that remain benign and localized to the prostate.

Prostate cancer is the second leading cause of cancer death in men, and an estimated 230,000 cases will be diagnosed this year, according to the American Cancer Society. Treatment for prostate cancer can cause significant side effects, including sexual and urinary dysfunction, yet may not be needed for men whose cancers are unlikely to spread.

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- Philip A. Beachy

The researchers' findings were published September 12, 2004, in an advance online publication in the journal *Nature*. The scientists were led by Howard Hughes Medical Institute (HHMI) investigator Philip A. Beachy and his colleagues at The Johns Hopkins University School of Medicine, Drs. David Berman and Sunil Karhadkar. Additional coauthors included other colleagues at Johns Hopkins and a researcher from the U.S. Department of Agriculture.

“These findings quite unexpectedly extend understanding of the Hedgehog pathway to a role in prostate cancer, which is a major form of cancer,” commented molecular oncologist Charles Sawyers, an HHMI investigator at the Jonsson Comprehensive Cancer Center at UCLA. “The results are incredibly interesting, because they are among the most promising I’ve seen to enable distinguishing good-risk cancers from bad-risk cancers - and thus, those that need minimal therapy from those that are lethal.”

The Hedgehog signaling pathway is a well-known regulator of organ development. Beachy and his colleagues, as well as researchers at other institutions, have found that in some cancers, this pathway has escaped the normal control mechanisms and helped spur uncontrolled cell proliferation. These include cancers arising in organs of the gastrointestinal tract, such as the stomach, pancreas, esophagus and biliary tract.

According to Beachy, since all these cancers arise in organs of endodermal origin, it seemed reasonable to test whether activation of the Hedgehog pathway might similarly drive the development of prostate cancer, which also arises from endodermal tissues.

In their initial studies, Beachy and his colleagues established that the Hedgehog pathway was, indeed, active in cultures of human metastatic prostate cancer cell lines. Blocking Hedgehog signaling with cyclopamine, a drug discovered by Beachy's group that targets another protein in the pathway, *Smoothed*, inhibited growth of these cell lines. Furthermore, they showed that when the tumor cells were introduced into mice, cyclopamine caused a permanent regression of the tumors.

“We interpreted the finding in mice to mean that we had probably killed tumor stem cells responsible for propagating the cancers,” said Beachy. “This finding led us to explore the role that the Hedgehog pathway might play in the function of normal progenitor cells.”

To study the relationship of the Hedgehog pathway to normal prostate stem cells, the researchers performed experiments in mice in which they eliminated the male hormone, androgen, causing regression of the prostate. Normally, restoring androgen causes the prostate to regenerate. However, the scientists were able to block this regeneration by giving the animals cyclopamine or a Hedgehog-neutralizing antibody.

In additional studies of cultures of cells that closely resemble these prostate progenitor cells, the researchers found that switching on the Hedgehog pathway caused them to proliferate and form tumors when implanted into mice.

“This was a very striking observation, because it's very tough with manipulation of expression of a single cellular gene - and has never been done before, to my knowledge - to cause a primary human cell to become a

cancer,” said Beachy. “And that suggests that perhaps we have identified the right prostate target cell and activated the right pathway to trigger cancerous growth.”

To relate their findings to the metastatic process, the researchers tested samples of metastatic prostate cancer from men who had died of the disease. They found a uniformly high level of Hedgehog activity in these tissues, compared to benign prostate tissue samples. The researchers also found high levels of Hedgehog pathway activity in rat prostate cancer cells known to be actively metastatic.

In contrast, cells that were not metastatic showed low levels of activity. In particular, said Beachy, their experiments showed that pathway activation in metastatic cancers depended on the expression of *Smoothened* - suggesting that gene may be “a focal point of regulation in tissue regeneration and tumorigenesis.”

They also found they could convert low-metastatic cells into highly metastatic tumors by activating the Hedgehog pathway. “We actually found we could interconvert the two kinds of cell lines in mice,” said Beachy. “Whereas the high-metastatic lines were normally rapidly lethal in the mice, we could prolong survival essentially indefinitely by giving them cyclopamine. And when we activated Hedgehog in the low-metastatic lines, they became highly lethal,” he said.

HHMI investigator Matthew Scott, a developmental biologist who has studied the Hedgehog pathway at Stanford University, noted that Beachy's “study is very thoroughly done and holds great promise in the search for new treatments for human disease. Quite possibly only some of the cells in a tumor - “tumor stem cells” - have the especially dangerous property of unlimited growth, so the search for ways to identify such cells is important,” he said.

According to Beachy, both diagnosis and treatment of prostate cancer could benefit from understanding the role of Hedgehog activation in carcinogenesis. “If clinicians could use Hedgehog activation - perhaps measured by detecting some marker in the blood - to distinguish indolent from metastatic disease, they could know to treat the metastatic form and not the indolent form,” he said. “If the indolent form, for example, were detected in older men, it might not be as necessary to perform a prostatectomy, since there would be little likelihood of metastasis.

“This finding also suggests that it may be possible to treat the metastatic disease with inhibitors of the Hedgehog pathway,” said Beachy. “However, given the broad importance of this pathway, there are questions of unwanted side effects. We have been able to give effective doses of cyclopamine to mice for long periods of time without any obvious detriment. But we have no idea whether there are long-term physiological effects, or whether humans

might suffer side effects that we could not detect in animals.”