Drug may prevent development of invasive bladder cancer, researchers say

October 14 2014, by Krista Conger

Philip Beachy and his colleagues found in a study of mice that a drug that suppresses the immune system may prevent invasive bladder cancer. Credit: Steve Fisch

A drug already approved for use in humans may prevent invasive bladder cancer, according to a study by researchers at the Stanford University School of Medicine.

The drug, FK506, is commonly used to suppress the immune system in organ transplant recipients to combat rejection.

The researchers found that low doses of FK506, also known as tacrolimus, prevented the development of invasive bladder cancer in 10 out of 10 laboratory mice that were given a carcinogen over five months.

In contrast, seven of nine control mice developed invasive cancers during the same time period.

"This could be a boon to the management of bladder cancer patients," said Philip Beachy, PhD, professor of biochemistry and of developmental biology at Stanford and a Howard Hughes Medical Institute investigator. "Bladder cancer is the most expensive cancer to treat per patient because most patients require continual monitoring. The effective prevention of progression to invasive carcinoma would be a major advance in the treatment of this disease."

Beachy is the Ernest and Amelia Gallo Professor in the School of Medicine and a member of the Stanford Cancer Institute and the Stanford Institute for Stem Cell Biology and Regenerative Medicine. He is the senior author of the study, published Oct. 13 in *Cancer Cell*. Former postdoctoral scholar Kunyoo Shin, PhD, and former graduate student Agnes Lim, PhD, are the lead authors.

Two types of bladder cancer

Bladder cancer is the fourth most common cancer in men and the ninth most common in women. Smoking is a significant risk factor. There are two main types of the disease: one that invades the muscle around the bladder and metastasizes to other organs, and another that remains confined to the bladder lining. The noninvasive type, which comprises about 70 percent of all bladder cancers, is treatable. The invasive form is largely incurable and often deadly. It is also expensive and difficult to treat, and the high likelihood of recurrence requires ongoing monitoring after treatment.

Some noninvasive cases will progress to invasive cases. FK506 works, the researchers found, by activating a molecular pathway that signals potential cancer cells to become specialized, nondividing tissue. This

keeps them from engaging in the uncontrolled growth that can lead to the invasion of surrounding tissue.

Beachy and study co-authors Joseph Liao, MD, associate professor of urology at Stanford and chief of urology at the Veterans Affairs Palo Alto Health Care System, and Edda Spiekerkoetter, MD, an assistant professor of pulmonary and critical care medicine, now are seeking funding to conduct clinical trials of FK506 on people with localized bladder cancers to learn if the drug can also delay progression of the disease in humans.

How bladder tissue is organized

Researchers in Beachy's laboratory have been studying the bladder, its development and its highly organized structure for several years. The bladder's inner lining is made up of a tightly connected layer of umbrella cells, which protects the underlying cells from toxins and waste in the urine. Under them are intermediate and basal epithelial cells (together these umbrella, intermediate and basal cells make up the urothelium), and then a nonepithelial layer of cells called the stroma. The stroma is separated from the urothelium by a thin tissue called the basement membrane.

In 2011, Beachy and his colleagues identified a bladder stem cell in the basal layer capable of regenerating the entire bladder lining after injury by bacterial infection, and showed that this cell uses a signaling molecule called sonic hedgehog to "talk" to neighboring cells in the stroma. Earlier this year, they showed that this cell is also the exclusive source of invasive bladder cancer in their mouse model of the disease.

The cascade of signals initiated by sonic hedgehog is called the hedgehog pathway, and this pathway is known to play a vital role in embryonic development and in many types of cancers in a wide variety of

organisms. Beachy identified the first hedgehog protein in fruit flies in 1992.

Although Shin and Beachy found, in their previous work, that sonic-hedgehog-expressing stem cells were absolutely necessary for the development of invasive bladder cancer in the mice, they were intrigued by the fact that expression of the hedgehog protein was invariably lost once the cells began to invade the surrounding stromal tissue. In other words, the development of invasive bladder cancers appeared to be a two-step process.

Provocative finding

"This was a very provocative finding," Beachy said. "It was clear that these bladder stem cells were the source of the intermediate cancers, or carcinomas in situ, that remain confined to the bladder lining. However, it was equally clear that sonic hedgehog expression must then be lost in order for those cancer cells to be able to invade surrounding tissue. We wondered whether the loss of this expression leads to increased tumor cell growth."

In the current study, Beachy and Shin collaborated with Liao to obtain human surgical samples for study.

"With his help, we were able to obtain samples of invasive bladder cancer tissue from human patients that were not contaminated with benign cells," Beachy said. The researchers confirmed that, like the mice in the previous study, the human cancer tissue had lost the ability to express sonic hedgehog.

They then bred a strain of laboratory mice that were unable to produce a critical protein in the hedgehog signaling pathway called Smoothened. Mice without Smoothened developed invasive cancers much more

quickly than their peers when a compound called N-butyl-N-4-hydroxybutyl nitrosamine, or BBN, was put in their drinking water. (Nitrosamines are carcinogens found in cigarette smoke; BBN is a type of nitrosamine that is specifically activated in the bladder.)

"We saw a striking effect when Smoothened was absent," said Beachy. "While control mice developed invasive bladder cancer after about six months of receiving BBN, some of the mice lacking Smoothened developed fully invasive cancers as early as three months."

Further investigation showed that sonic hedgehog signaling by cells in the bladder lining increased the levels of expression of a class of proteins called BMPs by the stromal tissue. These BMPs in turn stimulate the cells of the bladder lining to differentiate. But things go awry when sonic hedgehog expression is missing.

How cancer invades the bladder lining

"Wherever in the bladder lining that these cancer stem cells lose sonic hedgehog expression, there's a corresponding drop in the levels of BMP signals in the stroma," said Beachy. "That allows an invasion of cancer cells, which might usually be stopped in its tracks by the differentiation-inducing effects of BMP. With little or no BMP expression, that micro-invasion could persist, progress and form an invasive carcinoma that can kill you."

The researchers wondered whether artificially activating the BMP pathway could mimic sonic hedgehog signaling and prevent the development of invasive cancers in their mouse model. They knew, from the work of co-author Spiekerkoetter that low doses of FK506 can activate the BMP signaling pathway. (Spiekerkoetter is currently testing whether FK506 treatment can help patients with pulmonary arterial hypertension, which is associated with a deficiency in BMP pathway

activity.

The researchers gave laboratory mice BBN in their drinking water for four months—long enough to cause carcinomas in situ, but not invasive bladder cancers. They then split the mice into two groups, each of which continued to receive BBN for an additional month. One group also received a low dose of FK506 during that final month, while the other group received a placebo. They then assessed the health of the animals.

They found that none of the 10 mice that had received FK506 developed invasive bladder cancer after a total of five months exposure to BBN. In contrast, seven of nine animals that received the placebo did develop an invasive form of the disease. Beachy is now hoping to work with his collaborators to begin trials of FK506 in human patients with bladder carcinomas in situ.

"Bladder cancer has one of the highest recurrence rates of all cancers and poses many challenges in its clinical management," Liao said. "These insights into the molecular mechanism of cancer progression in this clinically relevant animal model are truly exciting. As a urologist who cares for many bladder cancer patients, I feel the possibility of using an approved drug to curtail progression of early stage cancer to muscle-invasive disease, which typically requires complete removal of the bladder, could be a potential game changer."

Provided by Stanford University Medical Center