Molecular probe 'paints' cancer cells in living animals

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Researchers at the Stanford University School of Medicine have developed a molecular probe that sets aglow tumor cells within living animals. Their goal is to use the probe to improve the diagnosis and treatment of cancer and other diseases.

The probe's main ingredient is a molecule that labels active proteases - protein-destroying enzymes - that run amok in cancerous cells. The molecule is normally invisible to the naked eye but it carries a fluorescent tag that lights up when it binds to the protease. The tag beams out near-infrared light that passes through skin and is detectable with a special camera. The use of the imaging technique in mice is described in a study to be published in the Sept. 9 advance online issue of *Nature Chemical Biology*.

"Nowadays the detection of cancer, breast cancer for instance, is normally done by mammography, using X-rays - which might actually increase your risk of cancer. We think these probes may ultimately provide a less harmful, noninvasive method of detecting cancer," said the article's lead author Galia Blum, PhD, a postdoctoral scholar in the laboratory of Matthew Bogyo, PhD, assistant professor of pathology.

And that's just for starters.

"It's neat. The next generation of our experiments will apply the probes during surgery," said Bogyo, the study's senior author. "It would be nice to 'paint' it on tissues so you could distinguish between tumor and non-

tumor."

A key advantage of this enzyme-targeting molecule is its size. About 100 times smaller than other molecular imaging reporters, it can easily slip across the cell membrane and enter living cells. It can also move through the animal quickly, which opens up the possibility of using the technique to light up tumors while surgery is in progress.

"Unlike other enzyme-targeting molecules, it's very specific, sticks to where it binds and does it all very rapidly - in 30 minutes or less," Bogyo said.

And unlike most other molecular probes, this type identifies only active enzymes. "We went one step beyond just telling if the enzymes are there. We can answer the question, 'Are they active"' That's important because an accumulation of inactive enzymes doesn't necessarily indicate disease," Blum said.

Bogyo, Blum and colleagues designed the probe to bind to a subset of a family of proteases called cysteine cathepsins, which are more active in several types of cancer as well as other diseases. Now they are tinkering with the probe's configuration in an effort to create a variant that recognizes the enzymes involved in apoptosis, the process of cell death. This could ultimately allow researchers and doctors to visualize response to chemotherapy in tumors, Bogyo said.

And because other diseases besides cancer involve hyped-up proteases - such as Alzheimer's, arthritis, atherosclerosis and osteoporosis - the approach might be of use in diagnosing and treating them as well.

The work went surprisingly smoothly because of Blum's background in chemistry as well as biology. Using her chemistry skills, she created the probes. Then she switched to biology mode and tested them. When she

discovered that an earlier version of the probe worked great in tissue culture but decomposed on contact with mouse blood, she was able to tweak the molecule's structure to survive inside a living animal.

In addition to the potential health-care applications, the approach provides a valuable research tool, the researchers said. "It allows you to see exactly where enzymes are active within living animals," said Bogyo.

The Stanford researchers' ultimate goal is to test it in humans, though they'll complete more testing in animals before requesting permission from the U.S. Food and Drug Administration to conduct a human trial. "Since there are currently no fluorescent imaging agents in use in humans, the approval process is likely to require significantly more preclinical data," Bogyo said.

In preparation, they are working with James Basilion, PhD, associate professor of biomedical engineering at Case Western Reserve University, who is using the probe in surgical procedures in animals. They are now testing the probe's ability to reveal the presence of glioma tumor cells during brain surgery in mice.

"Because glioma tumor tissue looks nearly identical to normal tissue, it's very difficult for surgeons to remove every last bit of it," said Bogyo. "We think this will help."

Source: Stanford University