New Technique Detects Enzyme Implicated in Cancer, Atherosclerosis

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The technique Manu Platt developed to detect mature cathepsin K in cells and tissues -- and not its immature form or one of the other 10 cathepsin varieties -- is more sensitive and less expensive than current, less reliable techniques. (Photo: Rob Felt)

(PhysOrg.com) -- A Georgia Tech research team has developed a new technique that reliably detects and quantifies an enzyme implicated in osteoporosis, arthritis, atherosclerosis, cancer metastasis and other disease processes.

An enzyme implicated in osteoporosis, arthritis, atherosclerosis and cancer metastasis -- cathepsin K -- eluded reliable detection in laboratory experiments in the past. Now, a research team at the Georgia Institute of Technology has developed an assay that reliably detects and quantifies mature cathepsin K using a technique called gelatin zymography.

"This assay is important because researchers and pharmaceutical companies need a dependable method for sensitively detecting a small amount of cathepsin K and quantifying its activity to develop inhibitors to the enzyme that can fight the diseases while minimizing side effects," said Manu Platt, an assistant professor in the Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University.

Cathepsin K is required to maintain adequate calcium levels in the body, but it can be highly destructive because it has the ability to break down bone by degrading collagen and elastin.

Platt described the cathepsin K detection protocol in the June issue of the journal *Analytical Biochemistry*. This research was funded by new faculty support from Georgia Tech, and the Facilitating Academic Careers in Engineering and Science Scholars (FACES) and Summer Undergraduate Research in Engineering (SURE) programs at Georgia Tech.

The benefits of this assay over existing techniques are numerous, according to Platt. The major advantage of this protocol, he said, is the definitive knowledge that mature cathepsin K is being detected in cells and tissues -- and not its immature form or one of the other 10 cathepsin varieties: B, H, L, S, C, O, F, V, X or W.

Another advantage of this technique is that it is more sensitive and less expensive than current, less reliable techniques. The new assay allows cathepsin K to be detected in quantities as small as a few femtomoles and does not require antibodies, which can be expensive and cannot be used across different species.

"In our experiments we were able to detect mature cathepsin K activity in quantities as small as 3.45 femtomoles with zymography, which was

10 to 50 times more sensitive at detecting the enzyme than conventional Western blotting," noted Platt, who is also a Georgia Cancer Coalition Distinguished Cancer Scholar.

In addition, zymography allowed the researchers to measure the activity of the enzyme, whereas Western blotting just measured its presence.

To detect mature cathepsin K with gelatin zymography, Platt and Georgia Tech undergraduate student Weiwei Li first separated the enzymes present in cells by their molecular weights. This allowed them to distinguish the mature form of cathepsin K from the immature form and other cathepsin varieties.

Then, to verify the identity and presence of mature cathepsin K, the team activated the enzymes in the gel. They created the perfect acidic environment for cathepsin K to thrive and added inhibitors to block the activity of all enzymes except mature cathepsin K.

To validate the cathepsin K activity detected in the laboratory experiments, Platt and Georgia Tech undergraduate student Zachary Barry developed a computational kinetic model of the enzyme's activity. By solving a system of differential equations, they were able to calculate the concentrations of immature, mature and inactive cathepsin K present at all times during the experimental procedure.

"It is more challenging to work with enzymes than proteins because enzymes have to be functional, which means they have to be folded correctly to be active," explained Platt. "The model suggested that even after the slight denaturation and refolding required by our assay, the cathepsin K activity determined by zymography reflected what happens in nature and was not an artifact of the experimental procedure."

The model also predicted something unexpected -- the inactive form of

cathepsin K commonly purchased from supply houses contained 20 percent mature enzyme.

"Cathepsins are implicated in many different diseases and the value of this assay is that it enables the measurement of previously undeterminable cathepsin activity in normal and diseased cells and tissues," noted Platt.

With this assay, Platt's team is currently investigating whether cathepsin K activity is different in the cells of individuals with metastatic and non-metastatic breast and prostate cancers, and the role of cathepsin K in cardiovascular diseases, such as stroke, in children with sickle cell anemia. They are also examining whether cathepsin K plays a role in the inflammation associated with HIV.

"This research should provide new information on a number of existing pathophysiological conditions where cathepsin K activity had been previously undetectable," added Platt.

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