Initial step to treat breast, prostate cancer

IIT Gandhinagar team finds biologically active protein yielded by E. coli to be effective

R. PRASAD

By using a smartly designed, customised *E. coli* vector, researchers at Indian Institute of Technology (IIT) Gandhinagar have successfully produced a large quantity (15 mg/ml) of a biologically active protein (human tousled-like kinase-1b or TLK1b) in pure form.

The reported yield for this protein using bacteria is only about 1 mg/litre. The protein is mainly involved in DNA damage response pathway in breast and prostate cancer cells.

The team led by Dr. Sivapriya Kirubakaran and Dr. Vijay Thiruvenkatam from the institute's Discipline of Biological Engineering found a handful of the 120 small molecules they had designed and synthesised were able to inhibit the protein.

Though the protein is produced in excess in cancer cells, the inhibition, which was as high as 75%, prevents the protein from repairing the DNA damage leading to death of cancer cells.

DNA gets damaged when cells are exposed to radiotherapy, chemotherapy or other environmental factors.

The researchers were able to get high yield of the protein by inserting a recombinant DNA into *E. coli*. The recombinant DNA has a gene that expresses both TLK1b protein and the bacteriophage lambda phosphatase protein.

Challenge: A handfulof small molecules were effective in inhibiting the protein, says Sivapriya Kirubakaran (second from left).

The simultaneous expression of both the proteins is responsible for the high yield," says Dr. Thiruvenkatam, one of the corresponding authors of a paper published in *Scientific Reports*.

In the absence of the bacteriophage lambda phosphatase gene, the TLK1b protein produced is not active as it remains insoluble. "The lambda phosphatase gene removes the phosphate so we were able to get the pure, active protein. Getting pure, active protein from bacteria has been a challenge so far," says Dr. Kirubakaran, the other corresponding author.

Since the yield has been much more than expected, the researchers are testing the bioactivity and the 3D crystallographic structure of the protein. "There is limited information on this protein's expression. So we intend studying the protein function and mechanism biochemically and structurally," she says.

The team has already biochemically confirmed that the protein produced by the bacteria is active. The next task is to know the precise structure the protein takes inside the cells. "Knowing the crystal structure of the protein will help in identifying the mechanism of DNA repair. It will help us in knowing the amino acid sequence that is responsible for DNA repair," he says.

Though a handful of small molecules were found to be effective in inhibiting the protein, the researchers are yet to study the mechanism of inhibition. "By knowing the crystal structure of the protein we can tell the sites where the small molecules get attached leading to inhibition. It will also help us design better small molecule inhibitors," Dr. Thiruvenkatam says.

"We are working on two other proteins in the DNA repair pathway which when inhibited using small molecules will increase the chances of cancer cell death. The inhibitors should be used along with chemotherapy or radiotherapy for best results," says Althaf Shaik, a co-author of the paper.

"The work we are currently doing is preliminary in nature. Our long-term goal is to know which functional groups on the small molecules needs to be modified to make them more specific so that they target the TLK1b protein present only in the cancer cells," says Siddhant Bhoir, first author of the paper.