Source : www.thehindu.com Date : 2018-09-30

IISC TEAM FINDS NEW APPROACHES TO KILL TB BACTERIA

Relevant for: Health, Education & Human Resources | Topic: Health & Sanitation and related issues

Protective protein : WhiB4 protein plays a crucial role in protecting TB bacteria from oxidation stress, says Amit Singh (right)

Oxidative stress can directly damage the DNA, proteins and lipids of most of the bacteria and eventually kill them. However, disease-causing bacteria have evolved mechanisms to survive such stressful conditions. One of the ways bacteria overcome oxidative stress is by condensing or compacting the DNA (nucleoid). Compacted DNA has reduced surface area and hence lower vulnerability to oxidative stress.

The role of several nucleoid-associated proteins produced by bacteria in condensing the DNA is also well known. But for the first time a protein (WhiB4) that condenses the DNA of TB-causing bacteria in response to oxidative stress has been found by a multi-institutional team led by Prof. Amit Singh from Indian Institute of Science (IISc), Bengaluru.

“Though the role of proteins in condensing DNA and the connection between DNA compaction and bacteria’s ability to survive oxidative stress are already known, this is the first time the role of a protein to condense DNA upon directly sensing oxidative stress in any bacteria has been reported,” says Prof. Singh. The results were published in the journal *Redox Biology*.

While DNA compaction helps the bacteria survive stressful conditions, the compaction has to be only for a brief period and should be reversible. Prolonged compaction could adversely impact bacterial multiplication, conversion of DNA into RNA, and formation of protein molecules.

The active form of WhiB4 protein is produced in the presence of oxidative stress leading to compaction of TB bacterial DNA. The protein level reduces after a while and thus preventing long-lasting condensation, and also allows the compacted DNA to revert to its original state. “We found the WhiB4 protein was helping the TB bacteria to persist within the host when exposed to stressful conditions,” says Dr. Manbeena Chawla, from IISc and one of the first authors of the paper.

Since prolonged DNA compaction would be detrimental to the bacteria and would eventually kill them, the researchers turned to genetic engineering to replace the promoter of the WhiB4 protein with another one that can be artificially regulated. (The promoter has to be active for a protein to be produced.) “Using extremely low dose (about 100 nanograms) of tetracycline drug we were able to artificially activate the promoter, which kept the WhiB4 levels elevated and guaranteed permanent DNA condensation,” says Dr. Saurabh Mishra from IISc and another first author of the paper.

“This was a proof-of-concept study to show that artificially elevating the levels of WhiB4 protein led to prolonged compaction of DNA leading to bacterial death which is oxidative stress dependent,” says Dr. Kushi Anand from IISc and another first author of the paper.

TB bacteria found inside human cells (macrophages) can be killed using two different approaches. One is to identify the negative regulator of WhiB4 protein and then developing an inhibitor against this regulator. The inhibitor will facilitate the production of WhiB4 protein in excess for a long time leading to long-lasting DNA compaction causing TB bacteria death. “But

we don’t know if there are one or more regulators of WhiB4 protein. Many times bacteria have multiple layers of backup system,” Prof. Singh says.

The second approach is to use a drug or an inhibitor specific to TB bacteria that directly causes DNA compaction for a long time leading to bacteria death. “Small molecules that compact *E. coli* DNA for extended period causing death has already been shown,” Prof. Singh says.

In any bacteria there are two pathways — production of antioxidants and DNA condensation — that help protect the bacteria from oxidative stress. In 2012 study in *Molecular Microbiology*, a team led by Prof. Singh had shown that WhiB4 protein is responsible for production of antioxidants. The latest study shows the role of WhiB4 protein in regulating DNA condensation. Thus WhiB4 protein plays a crucial role in protecting TB bacteria from oxidative stress.

Finding drugs that can either keep the WhiB4 protein level high or DNA condensed for a long time might be challenging. Particularly, as the drug has to cross the macrophage barrier and get inside the infected cell and then enter the TB bacteria. Alternatively, viruses that infect TB bacteria can be used as vehicles to carry WhiB4 protein into the bacteria. Here, the WhiB4 protein from TB bacteria genome has to be amplified and cloned into the virus genome.

These viruses specifically target TB bacteria and so human cells will be spared. But like in the case of the drug, the virus with the WhiB4 protein in its genome has to cross the macrophage barrier to get inside the infected cell before infecting the TB bacteria. And that will again be a challenge.

END