Tiny tweezers and yeast help researchers show how cancer drug works

July 9 2007

The annoying bulges of an over-wound telephone cord that shorten its reach and limit a caller's motion help to explain why drugs called camptothecins are so effective in killing cancer cells, according to investigators at St. Jude Children's Research Hospital and Delft University of Technology.

Using a type of nanotechnology called magnetic tweezers as well as yeast cells, investigators showed that a camptothecin drug called topotecan kills cancer cells by preventing an enzyme, called DNA topoisomerase I, from uncoiling double-stranded DNA in those cells. Instead, the DNA becomes locked in tight twists, called supercoils, which bulge out from the side of the over-wound DNA molecule—much like the bulges in an over-wound telephone cord. If these supercoils accumulate and persist while the cell is trying to separate the two strands of DNA to make exact copies of the chromosomes during cell division, the cells will die.

Nanotechnology studies work at a scale of about 100 nanometers or less. For comparison, one nanometer is approximately 10 times the size of an atom; and 10 nanometers is one-thousandth of the diameter of a human hair.

In this first-of-its-kind study, researchers used the microscopic magnetic tweezers to monitor changes in the length of an individual DNA molecule caused by the action of a single topoisomerase I enzyme; and to study how the binding of a single topotecan molecule to this enzyme-DNA complex alters DNA uncoiling. Based on the results of those

studies, scientists developed the supercoil theory to explain the drug's ability to kill cancer cells, and then tested that theory in yeast cells. Their conclusion—that accumulation of DNA supercoiling kills the cells—provides a novel model for how topotecan works; and it provides insights into the drug's action that could help scientists in the clinical development of these agents. A report on this work appears in the advanced, online issue of *Nature*.

"This is the first time that the tools of nanotechnology have helped scientists to develop a biological hypothesis that was subsequently tested by follow-up experiments in a living organism," said Mary-Ann Bjornsti, Ph.D., a member of the St. Jude Department of Molecular Pharmacology.

Delft University nanotechnology researchers in the laboratory of Nynke Dekker developed the magnetic tweezers for studies in biophysics and adapted the technique to the current study on the effect of topotecan on topoisomerase I in cooperation with Bjornsti, a co-author of the "Nature" report.

DNA is a double-stranded molecule resembling a flexible ladder. The sides of the ladder are backbones that hold half of each rung of the ladder. The entire molecule is twisted, somewhat like a flexible telephone cord.

Before cell division, a molecular machine unzips double-stranded DNA by slicing through the rungs of the ladder, separating the two strands into a wishbone-like shape called the "replication fork," Bjornsti said. The separation of these strands is a critical step in the duplication of a cell's chromosomes, which must occur before a cell divides. However, this also increases tension in the DNA ahead of the fork, causing it to buckle into supercoils.

To allow the replication fork to keep unraveling the double-stranded DNA, the cell uses the topoisomerase I enzyme, which makes a temporary nick in the backbone of one of the two strands of super-coiled DNA. This allows the DNA strands to uncoil, which removes the supercoils so the replication fork can continue separating the two strands and synthesize exact copies of each chromosome. Topotecan exploits the binding of topoisomerase I to double-stranded DNA that occurs when the cell tries to separate these strands.

Researchers already knew that topotecan “poisons” topoisomerase by binding to both the enzyme and to the nicked, single strand of DNA. This traps topoisomerase in place, turning the topotecan-topoisomerase-DNA complex into a roadblock that prevents the replication fork from advancing.

“Until now conventional wisdom was that topotecan kills cancer cells simply because the replication fork collided with the trapped topoisomerase,” Bjornsti said. “Our study suggests that the positive supercoiling that accumulates ahead of the replication fork contributes to cell killing.”

The researchers made their discovery using the magnetic tweezers technique to attach one end of a double-stranded DNA molecule from a magnetic bead while securing the other end to a glass surface. They then rotated a tiny magnet over the top of the magnetic bead, which in turn rotated the bead holding the DNA, twisting the DNA into supercoils and shortening it to about one-seventh its original length.

When the team added topoisomerase to the DNA, the strand uncoiled to its original length within a few seconds. This suggested that the enzyme had nicked the supercoils, relieving tension and allowing the DNA to expand to its previous length. But in the presence of topotecan, the rate of DNA uncoiling due to topoisomerase was reduced 20-fold compared

to uncoiling without topotecan. However, the surprising finding was that drug binding slowed toposiomerase uncoiling of overwound DNA (positive supercoils) more than the rewinding of the strands of DNA that was underwound (negative supercoils).

“Our finding that topotecan preferentially slows the uncoiling of overwound or positively supercoiled DNA for such a long period of time, suggested that DNA supercoiling actually prevented the replication fork from advancing, which triggered cell death,” Bjornsti said. “We decided to test this model by studying the effect of camptothecin on DNA in yeast cells during the process of gene expression.” During gene expression, the DNA strands are separated so the cell can copy the genetic information into RNA—a process called transcription. RNA is a modified form of the gene that the cell uses to make the protein for which the gene codes.

Bjornsti’s team inserted rings of double-stranded DNA called plasmids into yeast cells to create a model for studying camptothecin’s effect. Topotecan is an analog or related drug of camptothecin. As in DNA replication, gene expression requires the unwinding of the DNA strands. However, instead of duplicating DNA, transcription machinery makes an RNA message, which is then “translated” into proteins. With gene transcription, the unwinding of DNA produces positive supercoils in front of the transcription machinery, while negative supercoils form behind it.

When the investigators added topoisomerase, the positive and negative supercoils disappeared at about the same rate, apparently because the removal of positive supercoils was balanced by a similar reduction in negative supercoiling. When scientests added the drug camptothecin, the positive supercoils were removed more slowly by topoisomerase I than the negative supercoils. This was strong evidence that camptothecin (or topotecan) poisoning of topoisomerase I preferentially triggers the

accumulation of positive supercoiling ahead of complexes that unwind DNA, such as the transcription machinery or replication forks.

However, camptothecin did not cause the accumulation of positive supercoils in yeast cells, expressing a topoisomerase that was resistant to the drug. This was further evidence that camptothecins, such as topotecan, kill cells by preventing topoisomerase from uncoiling positive supercoils.

Source: St. Jude Children's Research Hospital