2011
NCI Alliance for Nanotechnology in Cancer Annual Bulletin

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RNA Nanoparticles Deliver

from the University of Cincinnati CNPP, currently University of Kentucky

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For years, RNA has seemed an elusive tool in nanotechnology research. Although easily manipulated in the laboratory, RNA is susceptible to quick destruction in the body when confronted with a commonly found enzyme. "The enzyme RNase cuts RNA randomly into small pieces, very efficiently and within minutes," explains Peixuan Guo of the University of Cincinnati. But by replacing a chemical group in the macromolecule, Guo says he and fellow researchers have found a way to bypass RNase and create stable three-dimensional configurations of RNA, greatly expanding the possibilities for RNA in nanotechnology.

In their work, Guo and his colleagues focused on the ribose rings that, together with alternating phosphate groups, form the backbone of RNA. By changing one section of the ribose ring, Guo and his team altered the structure of the molecule, making it unable to bind with RNase and able to resist degradation. "RNase interaction with RNA requires a match of structural conformation," he explained. "When RNA conformation has changed, the RNase cannot recognize RNA and the binding becomes an issue." While previous researchers have shown this alteration makes RNA stable in a double helix, Guo says that they did not study its potential to affect the folding of RNA into a

three-dimensional structure necessary for nanotechnology.

After creating the RNA nanoparticle, Guo and his colleagues successfully used it to power the DNA packaging nanomotor of bacteriophage phi29, a virus that infects bacteria. "We found that the modified RNA can fold into its 3-D structure appropriately, and can carry out its biological functions after modification," says Guo. "Our results demonstrate that it is practical to produce RNase-resistant, biologically active, and stable RNA for application in nanotechnology."

Because stable RNA molecules can be used to assemble a variety of nanostructures, Guo says, they are an ideal tool to deliver targeted therapies to cancerous or viral-infected cells. "RNA nanoparticles can be fabricated with a level of simplicity characteristic of DNA while possessing versatile structure and catalytic function similar to that of proteins. With this RNA modification, hopefully we can open new avenues of study in RNA nanotechnology."

Guo's group has also tested the safety of RNA constructs in the delivery of therapeutics to targeted cells. This work, explained Guo, represents "two very important milestones in RNA nanotherapy. One problem in RNA therapy is the requirement for the generation of relatively large quantities of RNA. In this research, we focused on solving the most challenging problem of industry-scale production of large RNA molecules by a bipartite approach, finding that pRNA [packaging RNA] can be assembled from two pieces of smaller RNA modules." Guo discovered pRNA in a bacterial virus in 1987 and later demonstrated that this unique form of RNA can self-assemble into nanoparticles.

Guo and colleagues detail multiple approaches for the construction of a functional pRNA molecule containing small interfering RNA (siRNA). siRNA has already been shown to be an efficient tool for silencing genes in cells, but previous attempts have produced chemically modified siRNA that last only 15-45 minutes in the body and often induce undesired immune responses.

"The pRNA particles we constructed to harbor siRNA have a half-life of between five and 10 hours in animal models, are non-toxic, and produce no immune response," said Guo. "The tenfold increase of circulation time in the body is important in drug development and paves the way towards clinical trials of RNA nanoparticles as therapeutic drugs."

Guo says the size of the constructed pRNA molecule is crucial for the effective delivery of therapeutics to diseased tissues. "RNA nanoparticles must be within the range of 15 to 50 nanometers," he said, "large enough to be retained by the body and not enter cells randomly, causing toxicity, but small enough to enter the targeted cells with the aid of cell surface receptors."

Guo also said that to his knowledge, this is the first naked RNA nanoparticle to have been comprehensively examined pharmacologically *in vivo* and demonstrated to be safe, as well as deliver itself to tumor tissues by a specific targeting mechanism. "It suggests that the pRNA nanoparticles without a coating have all the preferred pharmacological features to serve as an efficient nanodelivery platform for broad medical applications," he noted.

This RNA construct work is detailed in a paper titled, "Fabrication of Stable and RNasae-Resistant RNA Nanoparticles Active in Gearing the Nanomotors for Viral DNA Packaging Engineering of Self-Assembled Nanoparticle Platform for Precisely Controlled Combination Drug Therapy," published in the January 2011 issue of *ACS Nano*. The therapeutic delivery work is detailed in two papers titled, "Assembly of Therapeutic pRNA-siRNA Nanoparticles Using Bipartite Approach" and "Pharmacological Characterization of Chemically Synthesized Monomeric phi29 pRNA Nanoparticles for Systemic Delivery," published in the journal *Molecular Therapy* in July 2011. ◆