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RNA Constructs Thread Translational Needle

RNA-Based Nanoparticles Ferry RNA Payloads Past Physiological Barriers

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Noncoding RNA plays a major role in gene expression and gene regulation, and its malfunction often results in abnormal cellular activity. This understanding led to development of treatment strategies that use RNA both as therapeutics targets and treatment agents.

“We predict that the next milestone in drug development history will be RNA drugs or drugs that target RNA,” says Peixuan Guo, Ph.D., director of the University of Kentucky’s Nanobiotechnology Center, and chair of the Gordon Research Conference on RNA Nanotechnology. “This year’s Gordon Conference is dedicated to finding cross-disciplinary approaches to RNA nanotechnology research.”

The intent of the conference is to promote transformative advances that will enable the diagnosis and treatment of diseases utilizing the unique modality provided by RNA-based nanoparticles. Below are just a few examples of the exceptional research and collaborations that are poised to bring forward the new generation of therapeutics.

Illuminating Cancer Pathways

MicroRNA (miRNA) regulates expression of more than 90% of all genes in the human genome. Consequently, dysregulation of miRNA expression contributes to pathogenesis of most, if not all, human diseases, including cancer. Somatic alterations that initiate the tumors result in alterations in miRNA, which in turn affect numerous other genes in the cascade.

“Cancer pathways and miRNA are inseparably linked,” says Carlo M. Croce, M.D., director of the Institute of Genetics at Ohio State University. “Since miRNA is often a downstream target of an initial tumorigenic event, we can often find that initial cause by studying patterns of miRNA expression.”

Dr. Croce’s team was the first to identify a cause of chronic lymphocytic leukemia (CLL). A chromosomal region, which is lost in 70% of CLL, contains two miRNA genes, miR-15 and miR-16. The team also demonstrated that these miRNAs are negative regulators of another gene in the cascade, BCL-2. In May 2014 AbbVie presented interim results from a Phase Ib clinical trial of ABT-199, an investigational BCL-2 selective inhibitor. Results showed an overall response rate of 84% in patients with relapsed/refractory CLL.

“In many types of human cancers, miRNAs are mapped to the chromosomal regions that are deleted or amplified,” continues Dr. Croce. “In these cases, it may not always be possible to develop a pharmaceutical solution for the miRNA itself. However, the miRNA’s downstream targets could be targeted.”

His team identified consistently dysregulated miRNAs in hepatocellular carcinoma (HCC), and it uncovered mechanisms that linked the altered miRNAs to cancer pathways. The knowledge framework allowed identification of a target affecting the cell cycle. Dr. Croce notes that in vivo results with a compound directed against the identified cell cycle genes appear promising. He emphasizes that a global view of the entire pathway is a key for finding targetable genes.

Dr. Croce’s current research focuses on the role of miRNAs in creating the tumor microenvironment. He hypothesizes that tumors shed microvesicles carrying miRNAs. When the vesicles fuse with immune cells, miRNAs alter their TNF and IL-6 gene expression, facilitating the microenvironment favorable for tumor metastasis. A similar microvesicle-mediated process may be a cause of cachexia, a wasting syndrome that often accompanies cancer. “Answering basic biology questions will help us to lay the foundation for future disease therapeutics,” concludes Dr. Croce.

Sustaining Tumor Suppression

“The RNA interference pathway can be harnessed to suppress expression of any gene, expanding the drug targets beyond what is accessible by antibodies and small molecules,” says Judy Lieberman, M.D., Ph.D., chair and senior investigator, Cellular and Molecular Medicine, Boston Children’s Hospital and professor, department of pediatrics, Harvard Medical School. “However, there are two potential bottlenecks to delivering siRNA into cells, crossing the plasma membrane and once in the cell, getting out of endosomes.”

This limitation has restricted development of siRNA-based drugs primarily to liver diseases. Finding suitable methods to deliver siRNAs to other tissues would expand siRNA applications to tissues other than the liver.

Dr. Lieberman explored chimeric RNAs composed of an aptamer, a short structured RNA sequence that has been selected for high-affinity binding to a cell surface receptor, fused to an siRNA for targeted gene knockout. One aptamer she studied binds to the HIV receptor CD4. Fused siRNAs were designed to silence either viral genes or CCR5, an HIV co-receptor. These chimeras effectively inhibited HIV infection in vivo in humanized mice and in human tissue explants.

“Antiretroviral drugs in the form of intravaginal gels are able to interrupt HIV transmission, but their effect does not last and requires topical application daily or just before sexual intercourse,” continues Dr. Lieberman. “CD4 aptamer-siRNA chimeras efficiently silence gene expression for several weeks in the mouse genital tract. Such lasting effect could reduce the number of applications and improve compliance with the treatment regimen.”

Dr. Lieberman hopes that this technology will be developed to prevent the spread of HIV in third world countries. Using a similar approach, her team developed an effective tool to suppress epithelial breast cancers and their tumor-initiating stem cells.

Most epithelial cancers and their stem cells express EpCAM tumor-associated antigen, which is currently an FDA-approved marker for monitoring metastatic breast, colon, and prostate cancers. EpCAM aptamer-siRNA chimeric molecules (AsiCs) mediate knockdown of PLK1, a gene required for mitosis.

Dr. Lieberman's group observed that their chimeras were taken up by EpCAM+ cancer cells in xenografted tumors, and that the tumors regressed. Moreover, AsiC-treated cancer cells were unable to form tumors, indicating that the cancer stem cells had been eliminated.

Dr. Lieberman emphasizes that siRNA-based cancer treatments might still require a combination approach to lessen the chances of developing drug resistance: "Luckily, the AsiCs are easy to manufacture, are not toxic, do not stimulate an innate immune response, and do not induce antibodies." Dr. Lieberman hopes to translate her inventions into clinical use by starting a company based on AsiCs.

RNA Scaffolds as Delivery Platforms

"RNA nanotechnology differs from other platforms for the delivery of RNA therapeutics in that the scaffold, targeting ligands, and therapeutic modules can be composed entirely of RNA," says Dr. Guo, Ph.D. In his pioneering studies, Dr. Guo established the concept of RNA nanotechnology. He showed that reengineered pRNA (packaging RNA from bacteriophage phi29) fragments can be designed to form multimeric RNA nanoparticles with defined size and structure.

Since then, his laboratory has constructed varieties of RNA nanoparticles with desired functionalities using technologically simple bottom-up self-assembly. The particles can be constructed using chemically synthesized pRNA loops, palindrome sequences, and junction motifs. The pRNA-based nanoparticles are homogenous, thermodynamically and chemically stable, nonimmunogenic, and nontoxic, and they can be easily functionalized with targeting RNA aptamers, RNA interference (RNAi) modules, and detection molecules.

The nanoparticle size falls into the "Goldilocks" zone. Smaller particles (less than 10 nm) are easily excreted, whereas the larger ones (over 100 nm) are nonspecifically cleared by lung macrophages and liver Kupffer cells.

"We have been able to construct RNA nanoparticles that have the ability to navigate across physiological barriers to specifically target xenograft tumors in mice without collateral damage to healthy organs," explains Farzin Haque, Ph.D., research assistant professor, University of Kentucky. "The pRNA platform is highly versatile. We can essentially custom-design RNA building blocks to assemble nanoparticles for targeting different types of cancers, each possessing its own unique microenvironment."

Dr. Guo's lab continues evaluating targeted delivery of RNAi therapeutics for cancer therapy using animal tumor models. The RNA nanoplatform is not restricted for cancer therapy. Most recently, Dr. Guo's lab showed that RNA nanoparticles can be delivered to the cornea and retina of the eye, and that the specificity of delivery to a particular cell type is dependent on the size and shape of RNA nanoparticles.

"Our nanoparticles show promise as carriers for treatments of macular degeneration; HIV and hepatitis B

infections; cancer; and other diseases," says Dr. Guo. "I believe that in the next few years RNA will become a cornerstone of drug development."

RNA Nanoparticles vs. Metastases

Dr. Guo's laboratory pursues an extensive set of collaborations creating specialized RNA nanoparticles for glioblastoma, colorectal, and epithelial cancers. Piotr Rychahou, M.D., research assistant professor, Markey Cancer Center, University of Kentucky, highlights the first successful proof-of-principle study that may just pave the way toward the imminent therapeutic application of RNA nanoparticles. In collaboration with Dr. Guo's team, he demonstrated that systemic injection of RNA nanoparticles specifically targeted tumor metastasis without accumulating in normal tissues.

"Metastatic colorectal cancer claims nearly 50,000 lives per year," says Mark Evers, M.D., director, Markey Cancer Center. "Metastases are typically spread through liver and lungs, and surgical resection is still the only reliable treatment method. However, in many cases where lymph nodes are affected, even surgery is no longer an option."

For a number of years, Dr. Evers' laboratory has been pursuing nanoparticle delivery for treatments of colorectal cancer. Dr. Rychahou explains that delivery strategies behind most nanoparticles rely on the so-called enhanced permeability and retention effect (EPR). The theory suggests that due to their chaotic growth, tumors and their vasculature are more porous and, therefore, readily permeated by nanoparticles.

"Unfortunately, when we tested multiple existing nanocarriers, we also found considerable accumulation in normal tissues adjacent to metastatic tumors," recalls Dr. Rychahou. "That is, until we tested RNA nanoparticles created by Dr. Guo's lab."

The initial experiments with xenograft models demonstrated precision targeting of folate-conjugated RNA nanoparticles to cancer tissues overexpressing folate receptors. However, the real test was to evaluate these nanocarriers in metastatic cancer models. The tumor microenvironment of metastasis is believed to be quite different from the microenvironment of subcutaneous xenograft tumors. Moreover, the microenvironment of liver metastasis differs from that of lung metastasis.

Using the metastatic cancer model, the team demonstrated precise homing of folate-RNA nanoparticles to metastasis of both liver and lung without accumulation in normal organ tissues. Using fluorescent imaging, Dr. Rychahou followed the particle distribution throughout the mouse body, and noticed rapid excretion of unconjugated nanoparticles.

"These were pivotal experiments to establish realistic potential of these nanoparticles to detect cancer cells, and to maximize therapeutic efficacy of anticancer treatments while minimizing their systemic effects," explains Dr. Rychahou. "Now that we established basic pharmacokinetic parameters, dosage, and treatment schedule, our next step is to use this platform to deliver miRNA or chemical drugs to metastatic cancer cells, something that has never been achieved before."

Creating an Effective Delivery System

"Despite some successes of RNAi therapy, improvements are needed in a variety of areas," suggests Anil K.

Sood, M.D., professor and co-director of the Center for RNAi and Non-Coding RNA at the University of Texas, MD Anderson Cancer Center. For example, delivery challenges include the tumoral localization of RNAi nucleotides and the intratumoral mobility, tumor cell uptake, and intracellular trafficking of nanocarriers.

Prospects for overcoming these challenges were discussed June 2014 in the journal Science Translational Medicine, where an article contributed by Dr. Sood's team noted that "multifunctional nanocarriers represent a major advance for cancer RNAi therapeutics, with early successes already observed in clinical trials." The article pointed out that "efforts continue toward improving potency, pharmacokinetic profiles, and biocompatibility of existing delivery carriers."

Dr. Sood and collaborators adapted dioleoylphosphatidylcholine (DOPC)-based nanoparticles to produce carriers suitable for siRNA therapy. These carriers combine many desirable characteristics. For example, DOPC nanoliposomes are both biocompatible and biodegradable, and they show excellent endosomal escape. Functional siRNA can be easily loaded into the DOPC nanocarriers.

The team zeroed on the EphA2 receptor from the ephrin receptor subfamily of the protein-tyrosine kinases. It is frequently overexpressed in solid tumors, and its suppression correlates with reduction in tumor volume. Upon the completion of target validation and preclinical toxicology studies, EPHARNA (EphA2-targeted siRNA) gained an IND approval from the FDA to enter a Phase I trial. Dr. Sood is also exploring other natural materials, such as chitosan, for siRNA delivery.

"To support a more straightforward regulatory path through the FDA, EPHARNA did not include a targeting moiety," says Dr. Sood. "However, we are pursuing aptamer-targeted delivery in the next generation of the product."

He utilized a unique screening approach to select for the best binding aptamers. An aptamer library was tested against endothelial cells derived from patients' tissues. Those aptamers that bound to the cancer-associated endothelial cells, but not to the normal cells, were sequenced and ran through additional rounds of selection. In the end, annexin A2 was chosen as a marker, specific to tumor blood vessels.

The aptamers contain another unique feature, sulfhydryl groups, or thiols, in their backbone. This chemical modification provides enhanced nuclease resistance. Chitosan particles functionalized with annexin A2 aptamers and loaded with siRNA are currently in preclinical studies.