A gut feeling for CD39

By Tim Fulmer, Senior Writer

Researchers have reported that enhancing CD39 activity in the gut could trigger a T<sub>reg</sub>-mediated immunosuppressive response to help treat inflammatory bowel disease. The researchers are now developing a soluble form of CD39 that they hope could become an infusible therapeutic to treat colitis and Crohn’s disease.

Multiple signaling pathways underlie the immunosuppressive function of T<sub>reg</sub>. One of those pathways involves the T<sub>reg</sub>-mediated generation of extracellular adenosine, which binds its receptor on the surface of proinflammatory T cells and decreases their development and proliferation (see Figure 1, “Targeting CD39 in inflammatory bowel disease”).

A team of researchers led by Simon Robson of the Beth Israel Deaconess Medical Center has been looking for ways to increase levels of extracellular adenosine at sites of inflammation to treat inflammation and autoimmune diseases. The group has focused on enhancing the activity of CD39, an enzyme on the surface of T<sub>reg</sub> convert ATP to adenosine.

Previous work by the group showed that genetic knockout of CD39 led to aberrant proliferation of proinflammatory T cells and failure to prevent allograft rejection in mice.

Now the researchers have extended those findings to autoimmune disease. In a mouse model of chemically-induced acute colitis, animals with Cd39 knockout had greater disease severity than controls that had colitis and expressed Cd39. The knockouts showed increased infiltration of leukocytes and thickening of the colon wall.

Next, the researchers attempted to reverse disease severity by administering soluble apyrase, an enzyme that has the same activity as CD39. Compared with controls, Cd39 knockouts receiving the enzyme showed no significant weight loss (<i>p</i>&lt;0.05), a proxy for colitis severity.

Finally, the group analyzed publicly available genomewide expression profiles of humans and identified a SNP (rs10748643) near the promoter of CD39 that was strongly linked to CD39 levels. The low CD39-expressing genotype was significantly associated with Crohn’s patients, whereas healthy controls were enriched for the high CD39-expressing allele (<i>p</i>=0.0006).

Thus, in both mice and humans, low CD39 expression correlated with severity of gastrointestinal autoimmune disease. The findings were published in the Proceedings of the National Academy of Sciences.
POSH SPICING

By Michael J. Haas, Senior Writer

North Carolina researchers have designed splicing factors that could help treat cancer and other diseases by selectively promoting or inhibiting the expression of specific isoforms of their target genes. The technology offers potential advantages over existing therapies that target gene splicing, including longer-lasting effects and the ability to hit any gene.

A single gene may express different isoforms of a protein depending on how the gene’s transcripts are assembled (spliced) into a single mRNA molecule. Moreover, splicing isoforms can play differing—even opposite—roles in cellular function. For example, the Bcl-XL gene codes for both a splicing isoform that is involved in normal mitochondrial apoptosis and an antiapoptotic splicing isoform that is upregulated in many cancers.

A dearth of tools for studying splicing mechanisms has made it hard to study the functional significance of splicing isoforms and thus discern which isoforms are potential drug targets. Additionally, it’s hard to predict the specificity that endogenous splicing factors have for their targets, making them difficult to manipulate for therapeutic uses.

An important enabling step for studying splicing came in 2006, when a pair of researchers at the National Institute of Environmental Health Sciences (NIEHS) reported in the Proceedings of the National Academy of Sciences that domains of the protein pumilio homolog 1 (PUM1) could be engineered to bind a gene target with good specificity.

This gave NIEHS researchers and colleagues from The University of North Carolina at Chapel Hill the idea to design splicing factors that used PUM1 domains to recognize their target sequences.

The resulting engineered splicing factors (ESFs) are fusion proteins with two components. The first is an arginine-serine-rich or a glycinerich domain that can promote or inhibit gene splicing, respectively. The second component is a PUM1 domain engineered to bind a specific eight-nucleotide sequence in the region of the target gene that codes for the splicing isoform of interest.

As proof of concept, the researchers designed an ESF targeting Bcl-XL and delivered it to human breast and lung cancer cell lines. The ESF induced the cancer cells to express more of the proapoptotic splicing isoform of Bcl-XL and made the cells more sensitive to apoptosis-inducing chemotherapeutics than untreated control cells.

Results were reported in Nature Methods.

The team was led by Zefeng Wang, assistant professor of pharmacology at UNC Chapel Hill, and included other UNC Chapel Hill researchers and Traci Hall, principal investigator at NIEHS and leader of the 2006 study reported in PNAS.

“This is a very elegant, imaginative and novel approach to build an artificial splicing factor from scratch,” said Ryszard Kole, SVP of discovery research at AVI BioPharma Inc. “They essentially create novel components of the cellular splicing machinery. By doing so, they mimic the effects that were previously achieved by targeting splice sites with antisense oligonucleotides.”

AVI is developing antisense agents, called phosphorodiamidate morpholino oligomers (PMOs), to treat Duchenne muscular dystrophy (DMD), Ebola virus and Marburg virus infections, and restenosis.

“There is a gap in the tools available to study the transcriptome,” said Vin Kotraiah, associate director of collaborative therapeutics at ExonHit Therapeutics Inc., the U.S. subsidiary of ExonHit Therapeutics S.A. “We can identify alternative splicing events but don’t have good tools to study the consequences of alternative splicing events in a controlled setting.”

ExonHit utilizes its SpliceArray technology to identify splicing isoforms involved in disease. The company is developing small molecule therapeutics targeting those isoforms to treat Alzheimer’s disease (AD), cancer and epilepsy, and has a deal with Allergan Inc. to develop therapeutics against targets identified by monitoring alterations in splicing isoforms to treat pain, neurodegeneration and ophthalmic indications.

GENETIC ADVANTAGES

Kole said the ESFs have both advantages and drawbacks compared with antisense molecules. On the plus side, he said, ESFs “could be delivered with lentiviral vectors and incorporated into the genome, and so could have longer-lasting or even permanent effects compared with antisense oligonucleotides.”

But he added that ESF technology would introduce a foreign protein into the body, creating the potential for an adverse immune response.

“The next logical step is to test the approach in vivo in appropriate animal models,” Kole told SciBX.

Kotraiah said ESFs might have two additional advantages over antisense agents or small molecules that target gene splicing.

“Antisense oligonucleotides are primarily used to block certain splicing sites,” he said. “ESFs are more versatile because they can block or promote splicing” on the target gene.

Comparing with small molecules, ESFs might have a broader range of gene targets, noted Kotraiah. “It might be difficult to target every alternative splicing event with a small molecule that interacts with a protein upstream in the splicing pathway,” he said. “If this technology works as the authors think it does, ESFs could directly target any splicing event.”

SPLICING MINING

Nevertheless, Kotraiah said ExonHit is more interested in the immediate potential of ESFs as tools to study splicing isoforms as drug targets rather than using ESFs as therapeutics.

Thus, he thought ESF technology and ExonHit’s SpliceArray platform could complement each other. “SpliceArray can identify alternative splicing events that occur in diseased versus healthy cells,” he said. “Then you could follow on with ESF technology to induce the expression of alternatively spliced isoforms in models and study their functions. Conversely, off-target effects of ESFs on alternative splicing and gene expression could be monitored on a transcriptome-wide scale using SpliceArrays.”

(Continues on p. 8)
Universal antidote for aptamers

By Kai-Jye Lou, Staff Writer

Researchers at the Duke University School of Medicine have developed a strategy to reverse the activity of therapeutic aptamers in situations in which rapidly turning off the drug’s effects is desirable, such as in cardiovascular surgery. The approach, which harnesses nucleic acid–binding polymers as the aptamer antidotes, could bolster the safety profiles of aptamers and give them a leg up over antibodies that go after the same targets.

Turning off the activity of an aptamer isn’t exactly rocket science. The molecules are strings of nucleic acids, so rationally designed oligos that complement the aptamer sequence can modulate the aptamer’s activity. Indeed, earlier work from Duke and Regado Biosciences Inc. had done just that.

Regado’s lead program is the REG1 Anticoagulation System, a two-component system composed of RB006, a single-stranded nucleic acid aptamer that binds to and inhibits Factor IXa, and RB007, a complementary nucleic acid that binds to and neutralizes RB006.

The catch is that designing an oligo to counteract an aptamer is not a cookie-cutter approach—a separate agent needs to be designed for each aptamer. This could be a costly endeavor, as each new control agent would also need to undergo testing along with its paired aptamer therapeutic.

To reduce the cost of developing control agents, the Duke researchers began a search for compounds that could reverse the effects of multiple aptamers in a sequence-independent manner, which led them to a class of polymers that target the backbone of the aptamers.

“The real eureka moment came when we realized that when we inject nucleic acid–based drugs into people, there normally aren’t other free nucleic acids outside of cells in the bloodstream,” said Bruce Sul-

lenger, chief of the Division of Surgical Sciences at the Duke University School of Medicine and director of the Duke Translational Research Institute. “We found that compounds that recognized a component of the nucleic acid backbone, as opposed to a nucleic acid sequence, can be used to control the effect of aptamers.”

Two of the polymers identified from a screen reversed the effects of eight different RNA aptamers. The researchers chose to focus on one of the polymers, β-cyclodextrin–containing polycation (CDP), as it had high binding affinity for the aptamers and is known to have low toxicity.

In pigs, CDP reversed the effects of an anticoagulant RNA aptamer within about five minutes.

Results were published in Nature Medicine. Sullenger was a senior author on the paper.

“The cyclodextrin approach is going to be a cheap and fast approach for reversing the effects of aptamer therapeutics,” said John Rossi, chair of the Department of Molecular Biology at the Beckman Research Institute at City of Hope. “The development of a universal approach to control the activity of aptamer-based therapeutics makes the clinical use of these compounds more interesting.”

“The ability to develop such agents without the need to develop more expensive customized antidote oligonucleotides would give aptamer-based therapeutics a decided advantage in terms of clinical development and safety,” said Robert Schaub, SVP of R&D at Archemix Corp.

“The work could address one of the key challenges that physicians face, particularly in the antithrombotic space. Once a physician deploys a therapeutic in a patient, he usually does not have the ability to recall the decision,” said Christopher Rusconi, SVP of discovery and preclinical development and CSO of Regado. “Regado has invested a lot in the need to clinically control the effects of therapeutics, and the Nature Medicine paper further validates this as an important aspect in personalized medicine.”

The aptamer antidote concept potentially could be applied to other types of nucleic acid–based therapeutics, he added.

(Continues on p. 9)