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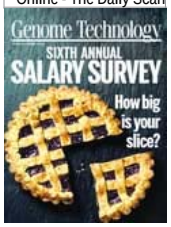

Emory Team Uncovers Small Molecule that Boosts RNAi Silencing; Effigene to Sell Tech

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By Doug Macron

Editor's Note: This article has been updated to correct an error regarding Effigene's intellectual property position.

A research team led by investigators from Emory University this week published data showing how a novel cell-based assay technology, licensed to startup Effigene Pharmaceuticals, was able to identify a small molecule that could enhance siRNA-mediated mRNA degradation and endogenous microRNA biogenesis.

Currently, Effigene is marketing a formulation of the compound, enoxacin, for improving siRNA silencing in eukaryotic cells. According to a company official, the firm also aims to strike alliances for using next-generation compounds with RNAi-based therapeutics, as well as for the assay technology itself.

Before it does so, however, Effigene plans to conduct a series of *in vivo* experiments with the support of grants from the National Institutes of Health to examine the effect of its various RNAi-enhancing agents combined with siRNAs in animal models of age-related macular degeneration, Huntington's disease, and Alzheimer's disease.

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"We know from having conversations with … a number of [undisclosed] pharmaceutical and biotechnology companies that they are very interested in seeing what we can do," Mark Ledden, president and CEO of Effigene, told *RNAi News* this week. "But they'd like to see one more round of *in vivo* testing" before they commit to the technology.

Ledden said that the NIH has indicated that it will award a phase I Small Business Technology Transfer grant to support the Huntington's disease work, although the exact size of the grant is not yet known. He added that Effigene investigators have also submitted a grant proposal for the AMD project and are in the process of putting together a proposal for the Alzheimer's disease effort.

'Relatively Non-Toxic'

Effigene was founded in late 2007 to commercialize the work of Emory researcher Peng Jin, who developed a chemical screen capable of identifying small-molecule enhancers of RNAi, Ledden said.

According to a paper published this week in *Nature Biotechnology* by Jin and colleagues from the University of Chicago, the Scripps Research Institute, the University of Texas Southwestern Medical Center, and Peking University, the screen uses a stable cell line derived from human embryonic kidney cells that express a gene encoding enhanced green fluorescent protein.

In experiments detailed in the paper, the cells were infected with a lentivirus expressing shRNAs targeting EGFP mRNA, which led to a reduction in EGFP levels. To confirm that the reduction in EGFP was due to an RNAi effect, the investigators transfected the cells with 2-O-methyl RNA, "which has been shown to block the activity of the lentivirus-encoded EGFP siRNA." The team observed an increase in GFP expression, they wrote.

They then isolated individual cell clones with moderate reductions in GFP expression and used them to identify small molecules that either enhanced or inhibited RNAi silencing.

After screening a library of 2,000 US Food and Drug Administration-approved compounds, the antibiotic enoxacin was identified for its ability to enhance RNAi silencing. "Enoxacin increased … gene knockdown mediated by siRNA against EGFP in our cell-based reporter system in a dose-dependent manner … whereas it had no effect on the cells expressing GFP only," they noted in the paper.

Importantly, enoxacin proved to be "relatively non-toxic," which is not surprising given the fact that the drug has been used for years to treat infections such as gonorrhea and urinary tract infections.

> *"Enoxacin increased … gene knockdown mediated by siRNA against EGFP in our cell-based reporter system in a dose-dependent manner … whereas it had no effect on the cells expressing GFP only."*

Enoxacin belongs to a family of synthetic antibacterial agents called fluoroquinolones. To test the specificity of the drug as an RNAi enhancer, other members of this family were tested, but only a handful triggered substantial increases in RNAi-mediated gene silencing, "suggesting that the RNAi-enhancing activity [of enoxacin] does not depend on general fluoroquinolone activity, but rather on the unique chemical structure" of the drugs, according to the *Nature Biotechnology* paper.

An examination of genome-wide gene expression in the cell lines tested by Jin and his colleagues also revealed "very few genes" with altered expression following enoxacin treatment, and none of the genes displayed "consistent and substantial changes," indicating that the RNAi-boosting effect was not due to pleiotropic effects of enoxacin, the team noted.

In regards to enoxacin's effect on endogenous miRNAs, the investigators wrote that they monitored the profiles of 157 miRNAs in transfected kidney cells stably expressing the primary transcript of miR-125a. Although the majority of miRNAs were unaffected, 13 had approximately two-fold increases in expression of their mature form.

"We also found decreased levels of the primary and precursor forms of miRNAs whose mature forms increased in the presence of enoxacin," suggesting that the compound could promote pre-miRNA processing, they wrote.

A series of *in vitro* and *in vivo* analyses revealed that enoxacin's RNAi-enhancing activity is dependent on its ability to facilitate the interaction between trans-activation-response region RNA-binding protein and RNAs, the researchers noted in their paper.

"Furthermore, we found that enoxacin has no effect in an *in vitro* RISC-cleavage assay, which argues against the potential involvement of enoxacin in the step of mRNA-target recognition and cleavage," they wrote. "Rather, these results together suggest that enoxacin targets the step of RISC loading by enhancing the interaction between TRBP and RNAs," they added.

In 2005, researchers from the Friedrich Miescher Institute for Biomedical Research in Basel, Switzerland, and Canada's McGill University first reported the link between TRBP and RNAi, finding that TRBP interacts with Dicer and is required for "optimal RNA silencing mediated by siRNAs and endogenous miRNAs."

Old Drug, New Use

With these and other data in hand, Effigene has begun selling its formulation of enoxacin, called EFI-001, for research applications as a means to generate near-term revenue. Since the compound is off patent, Ledden said that the company did not need to license it and, in fact, has filed for patent protection on the use with siRNAs for both research and clinical applications.

The company's patent application, No. WO/2007/025187, is entitled "Compounds and Methods for Modulating the Silencing of a Polynucleotide of Interest." It specifically claims "a method for modulating the level of a target polynucleotide in a cell [by] administering to the cell an effective amount of at least one RNAi modulating compound," namely a quinolone.

But the real focus of the company is finding partners interested in licensing either its RNAi- and miRNA-enhancing compounds, which currently include two other agents that have proven to be more potent than enoxacin but are still undergoing testing, or its screening technology for use in identifying their own compounds.

"The really exciting stuff we're doing is heading toward clinical applications," Ledden said. "At the same time, we see that we have an opportunity to provide …a product [in EFI-001] that could help people in the labs right now" while generating revenues for Effigene.

Selling EFI-001 is "really a finger of the overall strategy and one that we don't see as hugely important to our long-term business," he said. "We don't see ourselves as a medical tools company … [but] it seemed like an obvious opportunity worth exploring."

Ultimately, Effigene is hoping to grow into a "neurodegenerative-focused company with a lot of technology that has to do with the modulation of [RNAi and miRNA] pathways," Ledden said. The company's RNAi enhancers and screening technology is a large part of that, he added, although there are a number of related efforts underway at the company about which he declined to comment.

There is also the possibility that Effigene could branch out into drug making by in-licensing and developing an siRNA or miRNA-modulating agent, he added, but this is an unlikely course for the company since "we see ourselves primarily as a partner with other [big pharma and biotech]

businesses.

“We think we have the opportunity to partner on microRNAs, on siRNAs, on mapping of the pathways, on figuring out methods of action … and developing clinical tests and therapies,” he said.

But such partnerships are still on the horizon, Ledden added. “We’ve been really focused on proof of concept and making sure we get the [NIH] funding to go forward with disease-specific *in vivo* tests,” which should start yielding data within a year, he said.

“We’re going to be at a different kind of inflection point in terms of partnerships with big organizations in about nine to twelve months,” Ledden said.