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Day vs. night in spinal cord injury

By Kai-Jye Lou, Staff Writer

Patients with chronic spinal cord injuries often develop spasticity, but the underlying mechanisms remain unclear. Now, a group from the **University of Alberta** has uncovered a receptor involved in the process: a constitutively active isoform of the serotonin (5-HT_{2C}) receptor.[1] Targeting the receptor could be tricky, though, because in addition to fostering spasticity, the receptor also promotes motor recovery from the actual injury.

One option could be to agonize the receptor during the day to help motor recovery and antagonize it at night so the spasticity doesn't interfere with a patient's ability to sleep.

In spinal cord injury (SCI), neurons below the injury site lose their supply of serotonin (5-HT) from the brain stem, which leaves these cells in an unexcitable state. This loss of motor neuron excitability is one factor that contributes to muscle paralysis in SCI.

Despite the lack of serotonin, some motor neurons spontaneously regain their excitability in the weeks or months following injury via unknown mechanisms. Although this helps the recovery of motor function, it also leads to spasticity because some inputs from the brain that normally would prevent excessive excitability are absent.

"Despite many years of research, there are still no definite theories on why spasticity develops in chronically injured patients," said Serge Rossignol, the Canada Research Chair on the Spinal Cord and a professor in the Department of Physiology and Neurological Sciences at the **University of Montreal**. "The exciting aspect of the current work is that the researchers focused on one neurotransmitter and one receptor and went through the basic pharmacological studies to link aspects of locomotor recovery and muscle spasticity to a change that happens in a specific serotonin receptor."

The Alberta group showed that expression of a highly constitutively active isoform of the serotonin (5-HT_{2C}) receptor (HTR_{2C}) in motor neurons is upregulated in chronic SCI. Unlike other isoforms, this isoform is highly active even in the absence of serotonin (*see* **Figure 1, "A molecular mechanism involved in recovery and spasticity"**).[2]

The group showed that upregulated expression of the isoform corresponded to increased cell excitability, which in turn contributed to both motor recovery and the emergence of muscle spasticity.

The researchers showed that post-transcriptional editing at a particular site on 5-HT_{2C} receptor mRNA was significantly lower in the motor neurons of rats with chronic SCI than in those of uninjured controls ($p<0.05$). This difference in editing efficiency corresponded to a fourfold increase in the expression of the unedited 5-HT_{2C} receptor isoform, which increased

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motor neuron excitability compared with that in uninjured controls ($p<0.01$ for both).

In a rat model of chronic SCI, intrathecal delivery of a selective 5-HT_{2C} receptor inverse agonist reduced tail spasms compared with delivery of saline control. In an investigator-led trial in chronic SCI patients, treatment with cyproheptadine, a marketed, nonselective 5-HT_2 receptor inhibitor, significantly decreased leg muscle spasms compared with vehicle ($p<0.01$).

Cyproheptadine is a generic drug approved to treat allergies and hay fever. About 30 years ago, cyproheptadine was also shown to reduce spasticity and improve locomotion in SCI. It is still being used off-label to treat muscle spasticity in SCI patients but has multiple side effects like increased food intake and weight gain. **Merck & Co. Inc.** markets a branded version of the drug under the name Periactin.

Although cyproheptadine is known to inhibit serotonin signaling through the 5-HT_2 receptors, including 5-HT_{2C}, it was unclear how such receptors contribute to the emergence of spasticity in chronic SCI.

The results were published in *Nature Medicine*.

"We show that this increased excitability in the spinal cord is caused by serotonin receptors spontaneously turning on in the absence of serotonin," said David Bennett, co–senior author of the paper and a professor of rehabilitation medicine at the University of Alberta. "The challenge now is to design therapies to, on one hand, harness this spontaneous recovery of spinal cord function to help recover useful function and, on the other hand, turn off excessive spinal cord activity when it is not needed."

"Our work identifies one mechanism that seems to contribute to both recovery of locomotor activity and the development of spasticity," added Karim Fouad, co–senior author and professor of rehabilitation medicine at the university. "This suggests that we will have to be very careful when targeting the serotonin pathway to treat chronically injured patients for spasticity. We will need to make sure the drugs we give don't also block the recovery of locomotor function."

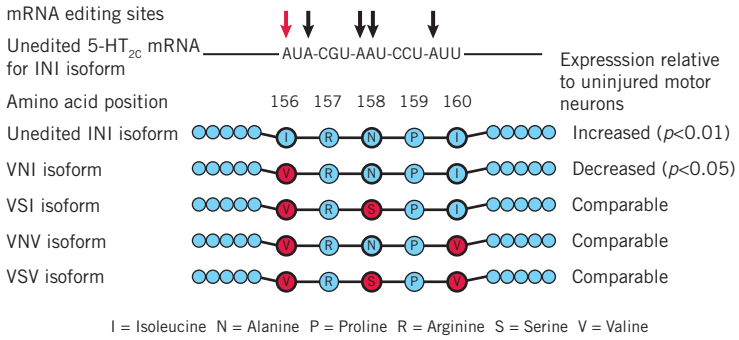

Figure 1. A molecular mechanism involved in recovery and spasticity. In *Nature Medicine*, researchers show that increased expression of a serotonin (5-HT_{2C}) receptor (HTR_{2C}) isoform with high constitutive activity mediates both the recovery of motor function and the emergence of muscle spasticity in chronic spinal cord injury (SCI).

Post-transcriptional editing is known to occur at five sites (arrows) on 5-HT_{2C} receptor mRNA. Edited mRNAs code for 5-HT_{2C} receptor isoforms that have less constitutive activity than receptors generated from unedited mRNAs.

Editing results in the substitution of different amino acids (red circles) at positions 156, 158 and 160 on the protein transcript. Receptor isoforms are named based on the amino acid present at these three positions.

The researchers showed that in chronic SCI, mRNA editing efficiency is decreased at one of the known edit sites on 5-HT_{2C} receptor mRNA (red arrow). This change in mRNA editing corresponds to decreased expression of the VNI isoform and increased expression of the INI isoform.

The researchers propose that this change in mRNA editing efficiency could be responsible for increased expression of the highly active INI receptor isoform, which in turn contributes to the increase in motor neuron excitability seen in chronic SCI.

"I thought this study was compelling in that it gives a rather convincing argument for receptors becoming constitutively active after spinal cord injury," said Vivian Mushahwar, an associate professor of cell biology at the university who was not involved with the current study. "This work implicates not only the receptor but also a particular change in the receptor that contributes to spasticity."

Molecular insights for new treatments

By shedding some light on the molecular pathways involved in the emergence of muscle spasticity following chronic SCI, the findings could provide a road map for developing therapies that enhance recovery but prevent spasticity.

"In chronically injured patients that want to walk during the day, you might be able to treat them with citalopram, which is an SSRI, to increase 5-HT_{2C} signaling, and then when the patients want to sleep, you could give them a drug that blocks 5-HT_{2C}, like cyproheptadine," said Fouad.

Citalopram is a generic selective serotonin reuptake inhibitor (SSRI). **H. Lundbeck A/S** and **Forest Laboratories Inc.** market the drug as Celexa to treat depression.

By blocking serotonin reuptake, more serotonin becomes available to activate its receptors—like the 5-HT_{2C} receptor.

The most widely used drug for spasticity is baclofen, a generic $GABA_B$ receptor agonist. "While the drug has a strong inhibitory effect, it also works nonspecifically, and patients can overdose and experience other negative side effects like hallucinations," Mushahwar told *SciBX*. "Maybe the doses of baclofen can be reduced considerably if it is combined with a drug that specifically targets the 5-HT_{2C} receptor. Targeted drugs may be able to control spasticity with a better side-effect profile."

Rossignol thinks the contribution of other pathways to the recovery of motor function and spasticity needs to be elucidated.

"After spinal cord injury, multiple pathways that provide the spinal cord with signaling molecules produced in other parts of the nervous system are lost," he told *SciBX*. "They have looked at serotonin receptors, but there are other receptors that might be changed as well when the spinal cord is injured, like GABA and noradrenergic receptors. Quite clearly, much more research is needed to understand what these and other receptors are doing after spinal cord injury."

Rossignol also wanted to know whether reducing spasticity impairs motor function recovery and how one would balance the use of serotonin receptor agonists and antagonists.

Fouad said the Alberta group is further dissecting the identified pathway to find ways to prevent excessive activity in motor neurons while also increasing recovery of motor function. He said it will be important to explore whether these receptors are involved in the functional recovery of the sensory system and whether other receptors can become constitutively active in chronic SCI.

Bennett added that the group also has an ongoing investigator-run trial evaluating the use of citalopram in chronic SCI patients.

The work is not patented.

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REFERENCES

1. Murray, K.C. *et al. Nat. Med.*; published online May 27, 2010; doi:10.1038/nm.2160
Contact: David Bennett, University of Alberta, Edmonton, Alberta, Canada
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2. Berg, K.A. *et al. J. Pharmacol. Exp. Ther.* **324**, 1084–1092 (2008)

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Common ground in autism and epilepsy

By Lev Osherovich, Senior Writer

A pair of studies has identified rare genomic copy number variants associated with both autism and epilepsy, suggesting there might be common mechanisms behind both diseases.[1,2]

The most immediate application of the findings could be to help in the early diagnosis and stratification of patients for deeper genetic studies. At the same time, the studies add support to the relatively recent hypothesis that these and other neuropsychiatric disorders like schizophrenia and mental retardation may have a common molecular basis.

Copy number variants (CNVs) are caused by DNA deletions, duplications or rearrangements scattered throughout the genome. They are usually benign but sometimes affect the activity of disease-related genes.

"Each one of us has several hundred CNVs. Some of these are common and you find differences among them in every individual, but some are rare and are more interesting from a disease perspective," said Heather Mefford, assistant professor of pediatrics and genetic medicine at the **University of Washington**. She was the corresponding author of the epilepsy study, which was published in *PLoS Genetics*.

> **"The crucial question is what is the potential link between autism and epilepsy. It's a puzzle that mutations in the same genes can cause very different clinical outcomes."**
>
> ***—Gudrun Rappold, Heidelberg University***

Analyzing CNVs can identify chromosomal abnormalities that are statistically linked with disease, thus pointing to potential disease risk genes. And because of the strong biological effects caused by gross chromosomal alterations like CNVs, their analysis "has been more effective at identifying loci than genomewide association studies," noted John Spiro, senior associate director of research at the **Simons Foundation Autism Research Initiative**.

Geraldine Dawson, research professor of psychiatry at **The University of North Carolina at Chapel Hill** and CSO of the patient advocacy group **Autism Speaks**, agreed that the new studies confirm the importance of rare CNVs, rather than common SNPs, in neuropsychiatric indications. Dawson was a senior coauthor of the autism CNV study, which was published in *Nature*.

The overlapping sets of genes associated with both diseases "begs the question of whether all psychiatric diseases are along a continuum and whether there's a polygenic nature to these disorders that manifests very differently depending on what cards you're dealt," said Robert Ring, senior director and head of **Pfizer Inc.**'s autism research unit.

"It's really impressive that these disorders have common genetic lesions," said Ruth Ottman, professor of epidemiology and neurology at **Columbia University**.

Ottman noted that although many patients with autism also have epilepsy, the reverse isn't true. Thus, she said, it's surprising so many epilepsy patients appear to have CNVs in genes previously associated with autism.

"The crucial question is what is the potential link between autism and epilepsy," said Gudrun Rappold, professor in and chair of the Department of Human Molecular Genetics at **Heidelberg University**. "It's a puzzle that mutations in the same genes can cause very different clinical outcomes."

"There could be a number of epilepsy-specific genes or autism-specific genes and you might have to reach a certain threshold to show a clear-cut clinical phenotype," said Rappold. "It's also possible that there could be a unique combination of mutations in each individual" that lead to specific clinical conditions.

"It's clear that there are overlapping symptoms and shared biology between autism spectrum disorders, schizophrenia and affective disorders," said Ring.

If there is a common mechanism behind many neuropsychiatric diseases, the new CNV studies may point to pathways that could be targeted to treat any combination of these indications. But until animal models of the CNVs identified in the studies appear, it's hard to predict which targets will be most readily druggable.

In addition, Spiro and Mefford noted that resequencing the candidate genes implicated by the CNVs may yet identify more subtle point mutations in the 90%–95% of patients in the two studies who did not carry large-scale chromosomal abnormalities. Alternatively, other genetic loci or perhaps environmental factors could prove more important in disease etiology for the majority of patients, requiring different therapeutic strategies for patients with and without CNVs.

Neuron theme in autism

The autism CNV study was done by an international consortium led by Dawson and Stephen Scherer, professor of medicine at the **University of Toronto** and director of The Centre for Applied Genomics at **The Hospital for Sick Children**.

Their team collected DNA from 1,275 children with autism spectrum disorder, and from each children's parents, and compared it with DNA from 1,981 healthy controls using a high-density microarray designed to detect CNVs. The team found 226 distinct CNVs that occurred in patients but not in controls. About 5.7% of autistic patients had at least one of the CNVs.

Many of the CNVs encompassed multiple genes, which prompted the researchers to compile a list of 149 candidate genes within the CNVs that had been linked previously to mental retardation or autism. These included genes for neuronal proteins involved in synaptic function.

"One of the common themes is the involvement of glutamate and the formation and function of synapses," said Dawson. She noted that the findings jibe with the theory that dysfunctional glutamate signaling underlies fragile X syndrome, a form of mental retardation that often is accompanied by autism.[3]

"This fits into our working hypothesis" about the importance of glutamate signaling in these disorders, said Randall Carpenter, president and CEO of **Seaside Therapeutics Inc.** "They find that relatively rare CNVs can converge on a few common signaling pathways" that have previously been linked to other neuropsychiatric indications like schizophrenia and fragile X syndrome.

Seaside's arbaclofen (STX209), a $GABA_B$ receptor agonist, is in Phase II testing for autism. The company also is developing STX109, a metabotropic glutamate receptor subtype 5 (mGluR5; GRM5) antagonist that is expected to start a Phase II trial for fragile X syndrome in early 2011. STX109 is partnered with **Merck & Co. Inc.**

Dawson said the findings should help focus the attention of drug developers on signaling pathways involved in synaptic structure and function. She said Autism Speaks is organizing a conference this year to connect pharma companies with academic researchers who are developing animal models of autism related to the discoveries described in the *Nature* paper.

"We can now talk about how to accelerate the science from genetic discoveries to target validation," she said.

The results are unpatented.

"We can now talk about how to accelerate the science from genetic discoveries to target validation."

—Geraldine Dawson, Autism Speaks

Disease nexus

Meanwhile, Mefford's team found that many CNVs previously linked with autism, mental retardation and schizophrenia are also associated with epilepsy.

Mefford and colleagues compared genomic DNA from 517 patients with idiopathic generalized and focal epilepsy with DNA from 2,493 healthy controls. Overall, 8.9% of the epilepsy patients had one or more CNVs that were likely to account for the disease.

Twenty patients had rearrangements in several neuropsychiatric CNV hotspots, including 15 patients with CNVs near the autism-associated gene *autism susceptibility candidate 2* (*AUTS2*).

Two other genes affected by CNVs described in Mefford's study, *neurexin 1* (*NRXN1*) and *contactin associated protein-like 2* (*CNTNAP2*), are also suspected autism susceptibility genes.

The most common CNV among epilepsy patients was in a region of chromosome 15 that contains the gene for nicotinic acetylcholine receptor α7 (CHRNA7). Mefford thinks abnormal activity of this acetylcholine receptor subunit could underlie a broad range of neuropsychiatric disorders.

Indeed, companies already are antagonizing CHRNA7 for a handful of indications. **Targacept Inc.** and **AstraZeneca plc** have TC-5619 in Phase II testing for attention deficit hyperactivity disorder (ADHD), schizophrenia and cognitive impairment. **Roche**'s MEM 3454 (RG3487) and **EnVivo Pharmaceuticals Inc.**'s EVP-6124 are each in Phase II testing for Alzheimer's disease (AD) and schizophrenia.

Mefford's next step is to make mouse knockouts that mimic the CNVs identified in her team's study. She has not patented her discoveries.

Psychotic markers

Researchers polled by *SciBX* agreed that the most immediate value of the two studies may be to help in the early diagnosis and stratification of patients for deeper genetic studies.

Although the specific genes altered by CNVs differed widely from patient to patient within each study, the findings nevertheless suggest that patients with similar CNVs can develop either epilepsy, autism or other neuropsychiatric disorders.

The more common of the CNVs identified in the study are "a valid basis for diagnostics and predictive screening," said Jonathan Sebat, assistant professor of psychiatry and director of the Beyster Center for Molecular Genomics of Neuropsychiatric Disease at the **University of California, San Diego**.

"You might hypothesize that children showing up in the clinic with deletions in some of these regions might be at risk for psychosis later in life and would be good candidates for early therapeutic intervention," Sebat said.

Dawson said that early detection and psychological therapy for autism patients can be critical in blunting the severity of behavior problems. However, she noted, because of the heterogeneous nature of the diseases caused by the CNVs, their utility as risk markers needs to be validated in prospective trials.

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REFERENCES

1. Pinto, D. *et al. Nature*; published online June 13, 2010; doi:10.1038/nature09146
   Contact: Stephen Scherer, The Hospital for Sick Children, Toronto, Ontario, Canada
   e-mail: stephen.scherer@sickkids.ca
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2. Mefford, H.C. *et al. PLoS Genet.*; published online May 20, 2010; doi:10.1371/journal.pgen.1000962
   Contact: Heather C. Mefford, University of Washington, Seattle, Wash.
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3. Kelleher, R.J. & Bear, M.F. *Cell* **135**, 401–406 (2008)

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Putting cancer in a PINCH

By Lauren Martz, Staff Writer

Although a combination of chemotherapy, radiation and surgery is one of the most widely adopted approaches to treating solid tumors, high resistance rates to chemotherapy and radiation therapy have spurred the search for ways to improve outcomes. Now, German researchers have found a way to increase the effects of both modalities by blocking PINCH1, a protein that is upregulated in cancer cells and is involved in their interaction with the surrounding extracellular matrix.[1] Whether PINCH1 itself or one of its downstream effectors is the best target will need to be elucidated next.

Interactions between cancer cells and their extracellular matrix, including adhesion and communication, are mediated by focal adhesions, which are multiprotein structures that can include integrins, growth factor receptors, cytoplasmic signaling molecules and adaptor molecules.

Besides their biomechanical function and role in sensing the cell microenvironment, focal adhesions also contribute to the survival of tumor cells by reducing their sensitivity to radiation or chemotherapy.[2]

Because they are large and heterogeneous, focal adhesions themselves are poor drug targets. Thus, a team at the **Dresden University of Technology** set out to determine whether molecules involved in regulating focal adhesion function might be better targets.

The team, led by Nils Cordes, team leader of the biological and molecular targeting group at the university's Center for Radiation Research in Oncology (OncoRay), chose to focus on PINCH1 (LIM and senescent cell antigen-like domains 1; LIMS1). This adaptor protein is known to be essential to focal adhesion regulation and to promote cell survival, spreading, adhesion and migration, albeit by unknown mechanisms.[3]

In a paper published in *The Journal of Clinical Investigation*, the team reported that *PINCH1* mRNA expression was higher in lung, colon, breast and prostate cancers than in normal tissues. PINCH1 levels were also higher in colorectal carcinoma biopsies than in normal colon tissue.

Cultured *Pinch1*$^{-/-}$ mouse embryonic fibroblasts had greater sensitivity to radiation than *Pinch1*-expressing cells. Fibroblasts lacking *Pinch1* were also more sensitive to cisplatin.

Those results carried over to both human cell lines and to mouse models of cancer. In animals with *Pinch1*$^{-/-}$ tumors, as compared to animals with *Pinch1*-expressing tumors, radiotherapy produced better reductions in tumor volume. Similarly, small interfering RNA–mediated *PINCH1* knockdown increased the sensitivity of human colon, lung, cervix, skin and pancreatic cancer cell lines to either radiation or the chemotherapeutics 5-fluorouracil (5-FU) and Gemzar gemcitabine.

Eli Lilly and Co. markets Gemzar to treat multiple cancers; 5-FU is a generic.

The Cordes group also teased out the mechanism by which PINCH1 increases tumor resistance: PINCH1 directly binds to and inhibits protein phosphatase 1 catalytic subunit α-isoform (PPP1CA; PP-1A), a protein responsible for regulating protein kinase B (PKB; Akt).

Akt can promote tumor resistance and progression.

In addition to researchers from Dresden University of Technology, the team included researchers from the **Max Planck Institute** and **Cancer Research UK**.

Point of intervention

As an adaptor protein, PINCH1 is capable of mediating protein-protein interactions between signaling molecules. But its drawback as a target is it lacks any enzymatic activity of its own.

> **"Therapeutic approaches blocking PINCH1 could be difficult to develop because the protein does not have kinase activity."**
> *—Ellen Van Obberghen-Schilling, Centre National de la Recherche Scientifique*

"Therapeutic approaches blocking PINCH1 could be difficult to develop because the protein lacks catalytic activity. Without catalytic activity, it is unclear how to block PINCH1," said Ellen Van Obberghen-Schilling, group leader of the Institute of Developmental Biology and Cancer at the **Centre National de la Recherche Scientifique** (CNRS).

"There are two pharmacological possibilities: the inhibition of PINCH1 expression, for example by siRNA, or the inhibition of the protein-protein interactions" between PINCH1 and PP-1A, said Joachim Fensterle, director of discovery and preclinical development at **Aeterna Zentaris Inc.**

However, Michael Teifel, senior director and head of preclinical development at Aeterna Zentaris, added that "targeting PINCH1 expression faces all current obstacles of siRNA therapies, including efficient delivery to achieve sustained expression. And targeting protein-protein interactions is still considered a demanding task."

Aeterna Zentaris' Perifosine, an alkylphosphocholine modulator of phosphoinositide 3-kinase (PI3K) and Akt, is in Phase III testing to treat colon cancer and multiple myeloma (MM).

Obberghen-Schilling said the next steps toward the development of tumor-specific protein-protein interaction inhibitors should be "to define the molecular structure of the protein-protein interface" between PINCH1 and PP-1A. She also noted that "PINCH1 interacts with other proteins as well, so it will be important to determine whether targeting this interaction has an effect on those interactions as well, for example its interactions involved in fibronectin matrix assembly."

Mary Lou Cutler, professor of pathology and director of the Molecular and Cell Biology Graduate Program at the **Uniformed Services University of the Health Sciences**, suggested a different route of attack—PP-1A itself. "The identification of PP-1A, a druggable target, provides another potential inhibitory point in the pathway," she said.

Tumor targeting

Although the *JCI* paper described overexpression of PINCH1 in various tumors, researchers contacted by *SciBX* were concerned that inhibiting its activity could lead to side effects because of the protein's involvement in survival of noncancerous cells.

"PINCH1 is ubiquitously expressed and involved in many developmental processes, including neuronal survival and neuronal signaling transmission," Fensterle noted. "Constitutive *Pinch1* knockout in mice leads to early embryonic lethality," although it's unknown what happens to adult mice with the target conditionally knocked out.

According to Obberghen-Schilling, the key for any PINCH1 modulator "will be to target the overexpression and not just the protein itself. It will be important to know in which cell populations within the tumor microenvironment PINCH1 is overexpressed."

The authors of the *JCI* article did not respond to interview requests.

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REFERENCES

1. Eke, I. *et al. J. Clin. Invest.*; published online June 17, 2010; doi:10.1172/JCI41078
   Contact: Nils Cordes, Dresden University of Technology, Dresden, Germany
   e-mail: Nils.Cordes@Oncoray.de
2. Hehlgans, S. *et al. Biochim. Biophys. Acta*. **1775**, 163–180 (2007)
3. Legate, K.R. *et al. Nat. Rev. Mol. Cell Biol*. **7**, 20–31 (2006)

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Pitting one virus against another

By Tim Fulmer, Senior Writer

Mount Sinai School of Medicine's Benjamin tenOever has devoted most of his research to developing vaccines for influenza and other RNA viruses. Now, his team has decided to put the influenza virus to work by turning it into a delivery vector containing microRNAs.[1] The goal is to 'infect' host cells with miRNAs that shut down the replication machinery of other viruses.

The group initially set out to answer the basic mechanistic question of whether RNA viruses such as influenza can produce functional miRNAs in the cells they target.

It is well known that DNA viruses like herpesvirus can synthesize miRNAs that not only regulate their own replication but also target the host transcriptional machinery. However, viral miRNAs produced by RNA viruses have not yet been detected, suggesting that RNA viruses may be unable to produce functional miRNAs.

> **"Their most valuable application may be in acute disease."**
> ***—Benjamin tenOever, Mount Sinai School of Medicine***

To shed light on the issue, tenOever's team engineered miR-124—a ubiquitous human miRNA involved in neuronal development—into the genome of the influenza A virus and looked at whether the modified strain would be able to deliver and express the miRNA *in vitro*.

In cultured epithelial cells, the modified influenza strain produced miR-124 at levels comparable to those of both an endogenous miRNA and miR-124 expressed by a standard plasmid.

The modified strain also showed growth and viral titers comparable to those of wild-type virus, demonstrating its ability to produce high levels of an miRNA without impairing its replication capacity in cell culture.

Next, the researchers looked at whether the miRNA produced by the modified virus could indeed silence expression of a target sequence within a cell. In cultured fibroblasts engineered to express a miR-124-targetable GFP, infection by the modified virus decreased the number of green fluorescent cells by 47.4% compared with no treatment.

Finally, in cultured neuronal precursor cells, which are known to undergo differentiation in the presence of miR-124, infection by the modified virus induced a neuron-like morphology compared with no infection. That result suggested that miR-124 expressed by the influenza virus functioned like endogenous miR-124.

The paper's authors concluded that influenza A virus and "other RNA viral vectors may be suitable delivery vehicles for RNA-based therapeutics." The findings were published in the *Proceedings of the National Academy of Sciences*.

"As proof of concept, the expression of exogenous miRNAs from an RNA virus is a very important finding, given that RNA viruses were previously thought to be incapable of expressing small RNAs such as miRNAs," said Andreas Bader, associate director of research at **Mirna Therapeutics Inc.**

Mirna's lead candidate is miR-34, a tumor-suppressing miRNA that is in preclinical development. The company plans to submit an IND in late 2011.

Virus vs. virus

tenOever told *SciBX* that because the modified influenza strains "produce transient, nontoxic spikes of miRNAs in the cells they infect, their most valuable application may be in acute disease."

Thus, his group now plans to use an miRNA-expressing influenza virus to treat an acute infection caused by another virus.

In this setting, the therapeutic miRNA could be designed to target a protein critical for the replication of a different virus. Host cells 'infected' by the influenza virus would produce high levels of the miRNA, which would subsequently block expression of the protein and prevent replication of the second virus.

"Our initial proof of concept will be aimed at using the modified influenza strain to deliver artificial miRNAs that inhibit vesicular stomatitis virus (VSV) simply because it is a safe and well-established viral model," said tenOever, who is corresponding author on the paper and an assistant professor of microbiology at the Mount Sinai School of Medicine.

If that shows promise, he said, the team then will look at other viruses, including respiratory syncytial virus (RSV) and parainfluenza virus.

Bader cautioned that much work is needed to demonstrate the usefulness of RNA viruses as delivery vectors. He said important questions include "whether miRNA expression by RNA viruses is universal or limited only to certain RNA sequences as well as how miRNA expression efficiency and *in vivo* safety of RNA viral vectors compare with more commonly used vectors like adenoviruses."

tenOever acknowledged that it also will be important to ensure that the influenza strain used to deliver the miRNA is properly attenuated to avoid causing an infection itself. "There are many ways of doing that," he said, including the use of influenza deletion mutants and additional miRNA-based modifications.

The Mount Sinai School of Medicine has filed a provisional patent application covering the findings described in *PNAS*. The IP is available for licensing.

Fulmer, T. *SciBX* 3(25); doi:10.1038/scibx.2010.754
Published online June 24, 2010

REFERENCES

1. Varble, A. *et al. Proc. Natl. Acad. Sci. USA*; published online June 7, 2010; doi:10.1073/pnas.1003115107
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COMPANIES AND INSTITUTIONS MENTIONED

Mirna Therapeutics Inc., Austin, Texas
Mount Sinai School of Medicine, New York, N.Y.

This week in therapeutics

THE DISTILLERY brings you this week's most essential scientific findings in therapeutics, distilled by *SciBX* editors from a weekly review of more than 400 papers in 41 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable.

This week in therapeutics includes important research findings on targets and compounds, grouped first by disease class and then alphabetically by indication.

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Autoimmune disease				
Autoimmune disease	*Sialic acid acetylesterase* (*SIAE*)	A genetic study suggests that defects in *SIAE* are associated with increased susceptibility to autoimmune diseases. In a cohort of patients and controls of European decent, genetic analysis identified loss-of-function *SIAE* mutations in about 3% of patients with various autoimmune diseases compared with in less than 0.5% of healthy controls. The *SIAE* mutations resulted in an odds ratio of 8.31, 7.89 and 8.62 for rheumatoid arthritis (RA) (p=0.0056), type 1 diabetes (p=0.0075) and any autoimmune disease (p=0.0002), respectively. Next steps include exploring how the defects contribute to autoimmune diseases. ***SciBX* 3(25); doi:10.1038/scibx.2010.755** **Published online June 24, 2010**	Patent pending covering diagnostic and therapeutic applications; available for licensing from the Massachusetts General Hospital Office of Corporate Sponsored Research & Licensing	Surolia, I. *et al. Nature*; published online June 17, 2010; doi:10.1038/nature09115 **Contact:** Shiv Pillai, Massachusetts General Hospital, Boston, Mass. e-mail: Pillai@helix.mgh.harvard.edu
Inflammatory bowel disease (IBD)	Tumor necrosis factor-α–induced protein 3 (TNFAIP3; A20)	Mouse studies suggest that increasing A20 expression in the intestinal epithelium could help treat IBD. Mice with intestinal epithelium–specific inactivation of A20 had greater susceptibility to colitis, intestinal cell apoptosis and infiltration of inflammation-inducing bacteria than wild-type littermates. Next steps include screening for compounds and peptides that increase the expression or activity of A20 to treat IBD. ***SciBX* 3(25); doi:10.1038/scibx.2010.756** **Published online June 24, 2010**	Findings unpatented; intestinal epithelium–specific A20 knockout mice available for licensing	Vereecke, L. *et al. J. Exp. Med.*; published online June 7, 2010; doi:10.1084/jem.20092474 **Contact:** Geert van Loo, VIB, Ghent, Belgium e-mail: geert.vanloo@dmbr.vib-UGent.be **Contact:** Rudi Beyaert, same affiliation as above e-mail: rudi.beyaert@dmbr.vib-UGent.be
Systemic lupus erythematosus (SLE)	Toll-like receptor 7 (TLR7); TLR9	A study in mice and humans suggests that TLR7 and TLR9 inhibitors could be useful for reducing the dosage of glucocorticoids used to treat SLE. In lupus mouse models and in samples from lupus patients, disease-associated proinflammatory plasmacytoid dendritic cells were more resistant to glucocorticoids in the presence of TLR7 and TLR9 activation than in the absence of their activation. Also in lupus mice, glucocorticoids plus a dual TLR7 and TLR9 inhibitor led to lower resistance to glucocorticoids and greater plasmacytoid dendritic cell death than glucocorticoids alone. Next steps include studying a TLR7 and TLR9 dual antagonist in a Phase I trial to treat lupus. DV1179, a dual TLR7 and TLR9 antagonist from Dynavax Technologies Corp., is in preclinical development to treat lupus and other autoimmune diseases. CPG-52364, a TLR7, TLR8 and TLR9 antagonist from Pfizer Inc., is in Phase I testing to treat SLE. IMO-3100, a dual TLR7 and TLR9 antagonist from Idera Pharmaceuticals Inc., is in preclinical development for SLE. ***SciBX* 3(25); doi:10.1038/scibx.2010.757** **Published online June 24, 2010**	Patent application covering paper's findings filed by Dynavax; GlaxoSmithKline plc has option to exclusively license IP	Guiducci, C. *et al. Nature*; published online June 17, 2010; doi:10.1038/nature09102 **Contact:** Franck J. Barrat, Dynavax Technologies Corp., Berkeley, Calif. e-mail: fbarrat@dynavax.com

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Various	B-cell lymphoma 2 (BCL-2; BCL2)	A study in mice suggests that the BCL-2 antagonist ABT-737 could be useful for treating autoimmune diseases. Mice treated with ABT-737, a research reagent from Abbott Laboratories, had lower T cell activity than mice given vehicle control. In the same mice, ABT-737 reduced the B cell response to an antigen and decreased pancreatic islet graft rejection compared with vehicle control. Next steps could include testing ABT-737 or other BCL-2 antagonists in mouse models of autoimmune and inflammatory disease. Abbott and Roche's Genentech Inc. unit are developing ABT-263, a BCL-2 antagonist that is in Phase I/II testing for chronic lymphocytic leukemia and small cell lung cancer. Five other companies have BCL-2 antagonists in various stages of development to treat cancer. ***SciBX* 3(25); doi:10.1038/scibx.2010.758** **Published online June 24, 2010**	Patent and licensing status undisclosed	Carrington, E.M. *et al. Proc. Natl. Acad. Sci. USA*; published online June 1, 2010; doi:10.1073/pnas.1005256107 **Contact:** David M. Tarlinton, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia e-mail: tarlinton@wehi.edu.au **Contact:** Andrew M. Lew, same affiliation as above e-mail: lew@wehi.edu.au
Cancer				
Breast cancer	SIN3 homolog A transcription regulator (SIN3A)	*In vitro* and mouse studies suggest that blocking interactions between SIN3A and its target transcription factors could help treat breast cancer. In mice and in human breast cancer cell lines, a decoy peptide of the SIN3A interacting domain (SID) prevented SIN3A from binding its targets and led to re-expression of silenced proteins and induction of cell death compared with a scrambled SID decoy peptide. In mouse models of breast cancer, transfection of cancer cells with a vector that expressed the decoy peptide led to impaired tumor growth compared with transfection using control vectors. Next steps include additional proof-of-principle studies of the SID peptide. ***SciBX* 3(25); doi:10.1038/scibx.2010.759** **Published online June 24, 2010**	Patent application filed; available for licensing	Farias, E.F. *et al. Proc. Natl. Acad. Sci. USA*; published online June 14, 2010; doi:10.1073/pnas.1006737107 **Contact:** Samuel Waxman, Mount Sinai School of Medicine, New York, N.Y. e-mail: Samuel.Waxman@mssm.edu
Cancer	*Activating transcription factor 7 interacting protein* (*ATF7IP*); *doublesex and mab-3–related transcription factor 1* (*DMRT1*); *telomerase reverse transcriptase* (*TERT*)	A genomewide study identified SNPs in *ATF7IP*, *DMRT1* and *TERT* that could help predict risk for testicular germ cell cancer. Genetic analysis of two cohorts of testicular germ cell cancer patients and healthy controls showed that SNPs rs4635969 and rs2736100 in *TERT* were significantly associated with disease risk (p=1.14×10^{-23} and p=7.55×10^{-15}, respectively). The study also showed that rs2900333 near *ATF7IP* and rs755383 near *DMRT1* were significantly associated with disease risk (p=6.16×10^{-10} and p=1.12×10^{-23}, respectively). Next steps include determining whether the candidate genes cause the disease and investigating the specific causal variants of the genes. ***SciBX* 3(25); doi:10.1038/scibx.2010.760** **Published online June 24, 2010**	Work unpatented; licensing status not applicable	Turnbull, C. *et al. Nat. Genet.*; published online June 13, 2010; doi:10.1038/ng.607 **Contact:** Clare Turnbull, The Institute of Cancer Research, Surrey, U.K. e-mail: clare.turnbull@icr.ac.uk

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer	Cysteine protease	*In vitro* studies suggest that a yeast-derived cysteine protease inhibitor could help treat or prevent cancer metastases. Bioassays of *Streptomyces spp.* broth identified a combination of two pentapeptides (CPI-2081) that inhibited the cysteine protease papain at low nanomolar concentrations. In cellular models of metastasis, CPI-2081 inhibited the migration of human breast cancer and melanoma cell lines compared with vehicle control. Ongoing and planned work includes chemical synthesis of CPI-2081 and testing the compound in animal models of cancer metastasis. ***SciBX* 3(25); doi:10.1038/scibx.2010.761** **Published online June 24, 2010**	Patent application filed by the National Chemical Laboratory and the National Centre for Cell Science; licensing status undisclosed	Singh, J.P. *et al. J. Med. Chem.*; published online June 16, 2010; doi:10.1021/jm9014179 **Contact:** Mohamad I. Khan, National Chemical Laboratory, Pune, India e-mail: mi.khan@ncl.res.in **Contact:** P.R. Rajamohanan, same affiliation as above e-mail: pr.rajamohanan@ncl.res.in
Cancer	IL-2 receptor; IL-2 receptor β-chain (IL2RB; CD122)	A study in mice suggests that preventing activation of the IL-2 receptor on lung endothelial cells could help avoid the pulmonary edema side effects associated with IL-2 immunotherapy for cancer. In a mouse model of cancer, a modified form of IL-2 that selectively activated the IL-2 receptor on leukocytes preserved the antitumor effects of IL-2 immunotherapy without causing vascular leakage side effects that lead to pulmonary edema. Next steps include humanizing the modified form of IL-2, which consists of the IL-2 ligand complexed to an antibody that targets the CD122 subunit of the IL-2 receptor. Nascent Biologics Inc. has multiple forms of the modified IL-2 ligand in preclinical development to treat cancer. Proleukin aldesleukin IL-2 from Novartis AG is marketed to treat melanoma and renal cancer. Ontak denileukin diftitox, an IL-2 plus diphtheria toxin fusion protein from Eisai Co. Ltd., is marketed to treat cutaneous T cell lymphoma (CTCL). ***SciBX* 3(25); doi:10.1038/scibx.2010.762** **Published online June 24, 2010**	Patent pending covering multiple forms of modified IL-2 ligand; licensed to Nascent Biologics from University Hospital of Lausanne; available for licensing from Nascent Biologics **Contact:** Mark Glassy, Nascent Biologics Inc., San Diego, Calif. e-mail: mark.glassy@aol.com	Krieg, C. *et al. Proc. Natl. Acad. Sci. USA*; published online June 14, 2010; doi:10.1073/pnas.1002569107 **Contact:** Onur Boyman, University Hospital of Lausanne, Lausanne, Switzerland e-mail: onur.boyman@chuv.ch
Cancer	LIM and senescent cell antigen-like domains 1 (LIMS1; PINCH1)	Studies of patient samples and of mice suggest that inhibiting overexpression of PINCH1 on tumors could help overcome radio- and chemo-resistance. In samples from human lung, colon, breast and prostate tumors, PINCH1 expression was significantly higher than that in healthy tissues ($p<0.0005$). In mice with *Pinch1*-deficient tumors, as compared to mice with tumors expressing *Pinch1*, tumor growth was delayed and recurrence-free survival was increased following radiation. Next steps could include assessing the potential side effects of targeting PINCH1 (*see* **Putting cancer in a PINCH, page 6**). ***SciBX* 3(25); doi:10.1038/scibx.2010.763** **Published online June 24, 2010**	Patent and licensing status unavailable	Eke, I. *et al. J. Clin. Invest.*; published online June 7, 2010; doi:10.1172/JCI41078 **Contact:** Nils Cordes, Dresden University of Technology, Dresden, Germany e-mail: Nils.Cordes@Oncoray.de

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer	Myeloid leukemia cell differentiation protein (MCL1; MCL-1)	*In vitro* assays and cell culture studies identified inhibitors of MCL-1 that could help treat cancer. *In vitro*, the MCL-1 BCL-2 homology domain 3 (BH3) helix was a potent inhibitor of MCL-1. In human leukemia and multiple myeloma (MM) cells, a hydrocarbon-stapled MCL-1 BH3 helix resulted in dose-dependent increases in sensitivity to proapoptotic agents compared with vehicle or proapoptotic agents alone. Next steps include evaluating the MCL-1-selective peptides in animal models. ChemGenex Pharmaceuticals Ltd.'s Tekinex omacetaxine mepesuccinate, a small molecule that targets the ribosome to inhibit synthesis of oncoproteins including MCL-1, is under review for treating chronic myelogenous leukemia (CML). The compound is also in Phase II testing for myelodysplastic syndrome (MDS). AT-101, a small molecule pan-inhibitor of the B-cell lymphoma 2 (BCL-2; BCL2) family of proteins from Ascenta Therapeutics Inc., is in Phase II testing for multiple cancers. ***SciBX* 3(25); doi:10.1038/scibx.2010.764** **Published online June 24, 2010**	Patent application filed; licensing status undisclosed	Stewart, M.L. *et al. Nat. Chem. Biol.*; published online June 20, 2010; doi:10.1038/nchembio.391 **Contact:** Loren D. Walensky, Dana-Farber Cancer Institute, Boston, Mass. e-mail: Loren_Walensky@dfci.harvard.edu
Cancer	*Polyribonucleotide nucleo-tidyltransferase 1* (*PNPT1*; *PNPASE*)	*In vitro* studies suggest that upregulating *PNPASE* could help treat cancer. In human melanoma cells, adenoviruses expressing *PNPASE* reduced levels of oncogenic *microRNA-221* (*miR-221*) compared with empty vector adenoviruses. Also in the melanoma cells, *PNPASE* knockdown or *miR-221* upregulation failed to block cancer cell growth. Next steps include conducting studies to determine the mechanism by which *PNPASE* degrades specific oncogenic miRNAs and testing upregulation of *PNPASE* in animal models of cancer. ***SciBX* 3(25); doi:10.1038/scibx.2010.765** **Published online June 24, 2010**	*PNPASE* gene patented by Columbia University; available for licensing discussions for certain applications	Das, S.K. *et al. Proc. Natl. Acad. Sci. USA*; published online June 14, 2010; doi:10.1073/pnas.0914143107 **Contact:** Paul B. Fisher, Virginia Commonwealth University School of Medicine, Richmond, Va. e-mail: pbfisher@vcu.edu
Cancer	Retinoid X receptor-α (RXRA; RXRα)	*In vitro* and mouse studies suggest that analogs of the NSAID sulindac could help treat cancer. In cultured cancer cells, sulindac bound to tumor-associated RXRα and induced apoptosis. In nude mice with cancer, a sulindac analog with strong affinity for RXRα increased inhibition of tumor growth compared with the parent compound. Next steps include preclinical studies of the analog to determine its pharmacological profile. Cancer Prevention Pharmaceuticals LLC's difluoromethylornithine (DFMO)/sulindac is in Phase III testing to treat colorectal cancer. Eisai Co. Ltd. markets Targretin bexarotene, which binds RXRα, to treat cutaneous T cell lymphoma (CTCL). NRX4204, an RXRα agonist from Vitae Pharmaceuticals Inc. and NuRx Pharmaceuticals Inc., is in Phase I testing to treat cancer. ***SciBX* 3(25); doi:10.1038/scibx.2010.766** **Published online June 24, 2010**	Patent filed covering methods for identifying compounds targeting RXRα and composition of analogs; sulindac analogs available for licensing	Zhou, H. *et al. Cancer Cell*; published online June 14, 2010; doi:10.1016/j.ccr.2010.04.023 **Contact:** Xiao-Kun Zhang, Sanford-Burnham Medical Research Institute, La Jolla, Calif. e-mail: xzhang@burnham.org

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer	Solute carrier family 31 copper transporters member 1 (SLC31A1; CTR1)	Patient sample and mouse studies suggest that copper chelators could help increase the efficacy of platinum-based chemotherapy. In tumor samples from ovarian cancer patients, low levels of the copper transporter *CTR1* were associated with poor clinical response to platinum-based therapies. In a mouse model of human cervical cancer, the chelator tetrathiomolybdate plus the platinum chemotherapeutic cisplatin reduced tumor size and impaired angiogenesis compared with either agent alone. Next steps include testing the combination therapy in other mouse models of cancer. Cisplatin is a generic cancer drug. ***SciBX* 3(25); doi:10.1038/scibx.2010.767** **Published online June 24, 2010**	Findings unpatented; licensing status unknown	Ishida, S. *et al. Cancer Cell*; published online June 14, 2010; doi:10.1016/j.ccr.2010.04.011 **Contact:** Douglas Hanahan, University of California, San Francisco, Calif. e-mail: dh@ucsf.edu
Head and neck cancer	Heat shock protein 27 (HSPB1; HSP27)	*In vitro* studies suggest that HSP27 inhibitors could help prevent metastasis in squamous cell carcinoma of the head and neck (SCCHN). A human metastatic SCCHN cell line showed higher levels of HSP27 and greater invasiveness than a primary SCCHN cell line derived from the same patient. In the metastatic cell line, HSP27 knockdown decreased invasiveness to levels that were comparable to those for the primary cell line. Ongoing work includes investigating the effects of Hsp27 knockdown in animal models of SCCHN. OncoGenex Pharmaceuticals Inc. and Isis Pharmaceuticals Inc.'s OGX-427, a second-generation antisense inhibitor of HSP27, is in Phase I testing to treat prostate, ovarian and breast cancers as well as other cancers. ***SciBX* 3(25); doi:10.1038/scibx.2010.768** **Published online June 24, 2010**	Unpatented; available for licensing	Zhu, Z. *et al. Mol. Pharm.*; published online June 11, 2010; doi:10.1021/mp100073s **Contact:** Duxin Sun, University of Michigan, Ann Arbor, Mich. e-mail: duxins@umich.edu **Contact:** Thomas Carey, same affiliation as above e-mail: careyte@umich.edu **Contact:** Xin Xu, Shandong University, Jinan, China e-mail: xinxu@sdu.edu.cn
Skin cancer	High mobility group box 1 (HMGB1); toll-like receptor 4 (TLR4)	A study in mice suggests that inhibiting a TLR4-dependent inflammatory response could help prevent skin cancer. In a mouse model of skin inflammation–mediated tumorigenesis, *Tlr4* knockout led to lower levels of proinflammatory cytokines and less recruitment of inflammatory cells than those in wild-type controls. Next steps include evaluating the role of the TLR4 ligand HMGB1 in inflammation-associated tumorigenesis. ***SciBX* 3(25); doi:10.1038/scibx.2010.769** **Published online June 24, 2010**	Patent and licensing status undisclosed	Mittal, D. *et al. EMBO J.*; published online June 4, 2010; doi:10.1038/emboj.2010.94 **Contact:** Maria Rescigno, European Institute of Oncology, Milan, Italy e-mail: maria.rescigno@ifom-ieo-campus.it
Cardiovascular disease				
Thrombosis	Integrin β_3 (GPIIIa; CD61)	Mouse studies identified an anti-GPIIIa antibody that could be a useful antithrombotic. In a mouse model of carotid artery thrombus, the anti-GPIIIa antibody prevented arterial platelet thrombi compared with saline and antibody control. In two different mouse models of stroke, the antibody protected against and reduced infarction without inducing hemorrhage compared with vehicle control. Next steps could include testing the compound in additional animal models of stroke. ***SciBX* 3(25); doi:10.1038/scibx.2010.770** **Published online June 24, 2010**	Patent and licensing status unavailable	Zhang, W. *et al. Blood*; published online June 4, 2010; doi:10.1182/blood-2010-01-264358 **Contact:** Thomas Wisniewski, New York University School of Medicine, New York, N.Y. e-mail: thomas.wisniewski@nyumc.org

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Endocrine disease				
Diabetes	Peroxisome proliferation–activated receptor-γ (PPARG; PPARγ)	*In vitro* and mouse studies identified a series of pyrimidine-5-carboxylic acid derivatives that could help treat diabetes. SAR studies identified a lead derivative with partial PPARG agonist activity. In a mouse model of diabetes, oral delivery of the derivative lowered plasma glucose to levels comparable to those seen using Avandia rosiglitazone. Next steps include comparing the lead with other marketed drugs in animal models. GlaxoSmithKline plc markets Avandia to treat diabetes. At least nine other companies have PPARG agonists in development stages ranging from clinical to marketed to treat diabetes. ***SciBX* 3(25); doi:10.1038/scibx.2010.771** **Published online June 24, 2010**	Findings patented; unavailable for licensing	Seto, S. *et al. J. Med. Chem.*; published online June 9, 2010; doi:10.1021/jm100443s **Contact:** Shigeki Seto, Kyorin Pharmaceutical Co. Ltd., Tochigi, Japan e-mail: shigeki.seto@mb.kyorin-pharm.co.jp
Infectious disease				
Candida	Glucosylceramide	*In vitro* and mouse studies suggest that blocking glucosylceramide biosynthesis could help treat *Candida albicans* infection. A mouse-based infectivity screen of a *C. albicans* knockout library identified 115 mutant *C. albicans* strains with lower virulence than wild-type strains. *In vitro* analyses of the virulence-defective strains showed that four of the knockout mutations disrupted genes in the biosynthetic pathway for glucosylceramide. Separate testing of the mutant strains in mice confirmed that glucosylceramide was essential to *C. albicans* infectivity. Future studies could include testing inhibitors of glucosylceramide or glucosylceramide synthase (GCS) in models of *C. albicans* infection. Genzyme Corp.'s eliglustat tartrate (Genz-112638), a ceramide analog that inhibits GCS, is in Phase III testing to treat Gaucher's disease. ***SciBX* 3(25); doi:10.1038/scibx.2010.772** **Published online June 24, 2010**	Patent and licensing status unavailable	Noble, S.M. *et al. Nat. Genet.*; published online June 13, 2010; doi:10.1038/ng.605 **Contact:** Suzanne M. Noble, University of California, San Francisco, Calif. e-mail: suzanne.noble@ucsf.edu
Tuberculosis (TB)	Not applicable	A study in mice suggests that lowering levels of *Mycobacterium tuberculosis*–specific T_{reg} cells could help treat TB. In a mouse model of TB infection, transplantation of *M. tuberculosis*–specific T_{reg} cells led to increased bacterial burdens in the lung compared with those in lungs of untreated controls. Next steps include determining whether exposure to environmental *Mycobacteria*, which share many antigens with *M. tuberculosis*, or immunization with TB vaccine bacillus Calmette-Guérin (BCG) is responsible for inducing antigen-specific T_{reg} cells that cross-react with *M. tuberculosis* and lower host immunity to the pathogen. ***SciBX* 3(25); doi:10.1038/scibx.2010.773** **Published online June 24, 2010**	Work unpatented; licensing status not applicable	Shafiani, S. *et al. J. Exp. Med.*; published online June 15, 2010; doi:10.1084/jem.20091885 **Contact:** Kevin B. Urdahl, University of Washington, Seattle, Wash. e-mail: kurdah@u.washington.edu **Contact:** Kiyoshi Takatsu, The University of Tokyo, Tokyo, Japan e-mail: takatsuk@med.u-toyama.ac.jp

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Musculoskeletal disease				
Bone repair	Fibroblast growth factor 9 (FGF9)	Mouse studies suggest that FGF9 could help accelerate bone healing. In mice with tibia injury sites, as compared with wild-type mice, *Fgf9* heterozygous knockout reduced bone regeneration and neovascularization. In the wild-type mice, exogenous FGF9 increased regeneration compared with saline buffer control. Next steps include testing recombinant human FGF9 in patients with long-bone fractures. ***SciBX* 3(25); doi:10.1038/scibx.2010.774** **Published online June 24, 2010**	Findings unpatented; unavailable for licensing	Behr, B. *et al. Proc. Natl. Acad. Sci. USA*; published online June 14, 2010; doi:10.1073/pnas.1003317107 **Contact:** Natalina Quarto, University of Naples Federico II, Naples, Italy e-mail: quarto@unina.it **Contact:** Michael T. Longaker, Stanford University School of Medicine, Stanford, Calif. e-mail: longaker@stanford.edu
Neurology				
Alzheimer's disease (AD)	Amyloid-β (A4) precursor protein (APP)	Mouse and *in vitro* studies suggest that L-3-*n*-butylphthalide (L-NBP) could help treat AD. In a mouse model of AD, oral delivery of L-NBP reduced glial cell oxidation and activation, decreased pathogenic β-amyloid (Aβ) plaque formation and improved cognitive impairment compared with delivery of vehicle. In cultured APP-expressing neuroblastoma cells, L-NBP promoted α-secretase cleavage of APP, suggesting that the compound shifts APP processing away from the generation of pathogenic Aβ plaques. Next steps could include clinical testing of the compound to treat AD or dementia. Raptor Pharmaceutical Corp.'s Posiphen, an APP inhibitor, is in Phase I testing to treat AD. ***SciBX* 3(25); doi:10.1038/scibx.2010.775** **Published online June 24, 2010**	Patent application filed covering use of L-NBP as antistroke or antidementia compound; licensed by CSPC Pharmaceutical Group Ltd.	Peng, Y. *et al. J. Neurosci.*; published online June 16, 2010; doi:10.1523/JNEUROSCI.0340-10.2010 **Contact:** C.A. Lemere, Harvard Medical School, Boston, Mass. e-mail: clemere@rics.bwh.harvard.edu
Alzheimer's disease (AD)	Ciliary neurotrophic factor (CNTF)	A study in cell culture and in mice suggests that antagonizing CNTF could help treat AD. In cell culture, recombinant CNTF reduced apoptosis in neurons exposed to pathogenic β-amyloid (Aβ) oligomers compared with vehicle control. Mice implanted with CNTF-secreting cells had better cognitive performance after exposure to Aβ than CNTF-free controls. Next steps could include testing the effect of CNTF-secreting implants in other transgenic mouse models of AD. Neurotech Pharmaceuticals Inc.'s NT-501, a formulation of encapsulated cells that secretes recombinant CNTF, is in Phase II testing for retinitis and age-related macular degeneration. ***SciBX* 3(25); doi:10.1038/scibx.2010.776** **Published online June 24, 2010**	Patent and licensing status undisclosed	Garcia, P. *et al. J. Neurosci.*; published online June 2, 2010; doi:10.1523/JNEUROSCI.4182-09.2010 **Contact:** Thierry Pillot, Nancy University, Vandoeuvre-lès-Nancy, France e-mail: thierry.pillot@ensaia.inpl-nancy.fr

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Epilepsy	*Nicotinic acetylcholine receptor* α_7 (*CHRNA7*)	A human genetics study suggests that CHRNA7 agonists could be useful for treating epilepsy. In a panel of patients with various forms of idiopathic epilepsy, about 10% had one or more copy number variants (CNVs) in genomic regions compared with healthy controls, which had no CNVs. About 10% of those individuals had deletions of a chromosomal region encompassing *CHRNA7*. Next steps include sequencing *CHRNA7* in more epilepsy patients and testing the effect of CHRNA7 agonists in animal models of epilepsy. CHRNA7 agonists in the clinic include Roche's MEM 3454 (RG3487) and EnVivo Pharmaceuticals Inc.'s EVP-6124, both in Phase IIb testing for Alzheimer's disease (AD), as well as Targacept Inc.'s and AstraZeneca plc's TC-5619, in Phase II testing for attention deficit hyperactivity disorder (ADHD; *see* **Common ground in autism and epilepsy, page 4**). ***SciBX* 3(25); doi:10.1038/scibx.2010.777** **Published online June 24, 2010**	Unpatented; licensing status not applicable	Mefford, H.C. *et al. PLoS Genet.*; published online May 20, 2010; doi:10.1371/journal.pgen.1000962 **Contact:** Heather C. Mefford, University of Washington, Seattle, Wash. e-mail: hmefford@u.washington.edu
Renal disease				
Polycystic kidney disease (PKD)	Glucosylceramide synthase (GCS)	Studies in human tissue samples and in mice suggest that inhibiting GCS could help treat PKD. In kidney tissue samples from PKD patients and in three mouse models of PKD, levels of the glycosphingolipid glucosylceramide were higher than those in normal tissues. In the mice, the GCS inhibitor Genz-123346 reduced kidney levels of glucosylceramide and the formation of kidney cysts compared with no treatment. Ongoing work includes investigating the role of other glycosphingolipids in PKD progression. Genzyme Corp.'s eliglustat tartrate (Genz-112638), a ceramide analog that inhibits GCS, is in Phase III testing to treat Gaucher's disease. Otsuka Pharmaceutical Co. Ltd.'s tolvaptan (OPC-41061), a vasopressin 2 (V2) receptor antagonist, is in Phase III testing to treat PKD. PLX5568 (R7376), a Raf kinase inhibitor from Plexxikon Inc. and Roche, is in Phase I testing to treat PKD. ***SciBX* 3(25); doi:10.1038/scibx.2010.778** **Published online June 24, 2010**	Patent and licensing status undisclosed	Natoli, T.A. *et al. Nat. Med.*; published online June 20, 2010; doi:10.1038/nm.2171 **Contact:** Oxana Ibraghimov-Beskrovnaya, Genzyme Corp., Framingham, Mass. e-mail: oxana.beskrovnaya@genzyme.com

This week in techniques

THE DISTILLERY brings you this week's most essential scientific findings in techniques, distilled by *SciBX* editors from a weekly review of more than 400 papers in 41 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable.

This week in techniques includes findings about research tools, disease models and manufacturing processes that have the potential to enable or improve all stages of drug discovery and development.

Approach	Summary	Licensing status	Publication and contact information
Chemistry			
Process-scale synthesis of chiral amines via enzyme biocatalysis for Januvia manufacture	Enzyme-catalyzed synthesis could help improve the manufacturing efficiency of diabetes drug Januvia sitagliptin. Docking and screening studies identified a variant of the bacterial enzyme ATA-117 that had 10,000-fold greater affinity for a Januvia precursor molecule than did wild-type ATA-117. The enzyme variant catalyzed the conversion of the precursor into Januvia with increased purity and yield, while reducing waste and overall cost, compared with the current manufacturing method. Merck & Co. Inc. plans to implement the method developed by Codexis Inc. for the full-scale manufacture of sitagliptin. Merck and Ono Pharmaceutical Co. Ltd. market the dipeptidyl peptidase-4 (DPP-4) inhibitor. ***SciBX* 3(25); doi:10.1038/scibx.2010.779** **Published online June 24, 2010**	Patent applications filed by Codexis; licensed to Merck	Savile, C.K. *et al. Science*; published online June 17, 2010; doi:10.1126/science.1188934 **Contact:** Christopher Savile, Codexis Inc., Redwood City, Calif. e-mail: christopher.savile@codexis.com **Contact:** Jacob M. Janey, Merck & Co. Inc., Rahway, N.J. e-mail: jacob_janey@merck.com
Drug delivery			
Transdermal drug delivery using carbon nanotube membranes	A transdermal drug delivery system using carbon nanotube membranes could provide a noninvasive approach to treating multiple diseases. In a proof-of-concept study, the system delivered nicotine through a patch of human skin at a rate of about 1.3 µmol/hr per cm^2 when activated and about 0.33 µmol/hr per cm^2 when inactivated. Those values are comparable to those for marketed nicotine cessation treatments. Next steps include evaluating the transdermal delivery system in animals. ***SciBX* 3(25); doi:10.1038/scibx.2010.780** **Published online June 24, 2010**	Transdermal drug delivery system patented; available for licensing from the University of Kentucky Commercialization & Economic Development Office	Wu, J. *et al. Proc. Natl. Acad. Sci. USA*; published online June 14, 2010; doi:10.1073/pnas.1004714107 **Contact:** Bruce J. Hinds, University of Kentucky, Lexington, Ky. e-mail: bjhinds@engr.uky.edu **Contact:** Audra L. Stinchcomb, same affiliation as above e-mail: audra.stinchcomb@uky.edu
Drug platforms			
Computational design of live attenuated influenza strains for vaccines	*In vitro* and mouse studies suggest that influenza strains containing suboptimal codon pairs could be useful for live attenuated influenza vaccines. Suboptimal codon pairs are modifications to the viral genome that cause impaired translation of viral proteins, resulting in poor viral replication compared with that of wild-type strains. In cell culture, a suboptimal influenza strain had a lower rate of growth than a wild-type control strain, indicating that the genetic substitutions led to attenuation. In mice, inoculation with the attenuated strain resulted in minimal virulence while eliciting an immune response that protected animals from influenza challenge. By comparison, all mice inoculated with a wild-type strain died. Next steps include studying the attenuated influenza strain in ferret models of influenza infection. FluMist, a live attenuated influenza vaccine from AstraZeneca plc's MedImmune LLC unit, is marketed to prevent influenza infection. ***SciBX* 3(25); doi:10.1038/scibx.2010.781** **Published online June 24, 2010**	Findings patented; available for licensing	Mueller, S. *et al. Nat. Biotechnol.*; published online June 13, 2010; doi:10.1038/nbt.1636 **Contact:** Steffen Mueller, State University of New York at Stony Brook, Stony Brook, N.Y. e-mail: smueller@ms.cc.sunysb.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Injectable polymer for repair of damage caused by myocardial infarction (MI)	A study in sheep suggests that an injectable polymer with adjustable biomechanical properties could be useful for treating MI. In a sheep assay of MI recovery, tissue injected with a hyaluronic acid hydrogel polymer with a high level of cross-linking showed stronger structural properties and smaller infarct size after eight weeks than tissue injected with a polymer with low levels of cross-linking. Next steps include developing an injection procedure for the hydrogel and testing its long-term efficacy in further preclinical studies. Myostratum LLC has a hydrogel in preclinical testing to treat MI. *SciBX* 3(25); doi:10.1038/scibx.2010.782 Published online June 24, 2010	Patent pending; nonexclusively licensed to Myostratum; available for licensing for other applications	Ifkovits, J.L. *et al. Proc. Natl. Acad. Sci. USA*; published online June 7, 2010; doi:10.1073/pnas.1004097107 **Contact:** Jason A. Burdick, University of Pennsylvania, Philadelphia, Pa. e-mail: burdick2@seas.upenn.edu
Markers			
Genetic risk markers for autism	A study in humans identified rare mutations that could be useful for predicting risk of autism. Microarray analysis of the genomes of autistic children and their parents identified a total of 226 gene deletions or duplications that were absent in healthy controls. Approximately 5.7% of autistic patients had at least one of these deletions or duplications. Next steps include resequencing the candidate genes from the study to identify point mutations that could correlate with disease in other autistic patients and developing screening procedures for early diagnosis and psychological therapy of autism (*see* **Common ground in autism and epilepsy, page 4**). *SciBX* 3(25); doi:10.1038/scibx.2010.783 Published online June 24, 2010	Unpatented; licensing status not applicable	Pinto, D. *et al. Nature*; published online June 13, 2010; doi:10.1038/nature09146 **Contact:** Stephen Scherer, The Hospital for Sick Children, Toronto, Ontario, Canada e-mail: stephen.scherer@sickkids.ca **Contact:** Geraldine Dawson, Autism Speaks, New York, N.Y. e-mail: gdawson@autismspeaks.org

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