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By Kai-Jye Lou, Senior Writer

Generating mAbs that target post-translational modifications such as phosphorylations could open up a host of new targets for cancer and other diseases associated with aberrant protein modifications, but the low immunogenicity of the modified sites has thus far stymied the use of conventional mAb-generation platforms.

University of California, San Francisco researchers have now developed a structure-guided phage display platform capable of producing mAbs that target post-translational modifications.<sup>1</sup> Next, the team will have to show that the mAbs target endogenous proteins *in vivo*.

Prior efforts to target post-translational modifications with mAbs usually involved animal immunization and the establishment of mAb-producing hybridomas. This approach is low throughput, takes months and has rarely yielded clones that produced mAbs that target phosphorylated amino acid residues.<sup>2,3</sup>

In addition, hybridomas often do not provide a limitless source for a mAb of interest because they can experience genetic drift. These cells thus require regular monitoring and testing to ensure they still produce the original mAbs.

Phage display-based approaches can rapidly generate mAbs but are even less efficient at generating mAbs that target post-translational modifications than animal immunization-based approaches.<sup>4</sup>

"Current phage display methods are not efficient for generating antibodies against peptides and phospho-peptides," said James Wells, chair of the Department of Pharmaceutical Chemistry and a professor in the Department of Pharmaceutical Chemistry and Department of Cellular and Molecular Pharmacology at UCSF. "This led us to explore the use of alternative antibody scaffolds with which to build our phage display libraries."

The UCSF group carried out a computational search of 60 known antibody structures to look for regions that might mimic a phosphate-binding pocket. The researchers identified one such pocket on a mouse Fab and built it into the complementarity-determining region (CDR) of a humanized Fab display scaffold.

Next, the team applied phage display mutagenesis to the parent scaffold to generate libraries of mAb scaffolds that specifically bound to phospho-serine, phospho-threonine or phospho-tyrosine.

The researchers sequenced the CDR regions of the mAb scaffolds and used *in vitro* assays and structural analysis to identify the best ones for each phospho-amino acid residue. They then used the lead

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phospho-serine and phospho-threonine scaffolds to build two phage display libraries of single-chain Fv mAbs.

Serine and threonine together account for nearly 100% of known phosphorylation sites, whereas tyrosine accounts for less than 0.05%.<sup>5</sup>

ELISA-based selection of the phage libraries identified 51 mAbs that selectively bound to phosphorylated peptides containing phospho-serine or phospho-threonine over nonphosphorylated counterparts, with affinities ranging from 42 to 5,000 nM. The peptides were 13–19 amino acids in length and derived from biologically relevant targets including protein kinase B (PKB; PKBA; AKT; AKT1).

Follow-up western blot analysis showed that a sample subset of the mAbs bound to the corresponding phosphorylated protein.

Results were published in *Nature Biotechnology*.

“Using an antibody scaffold that contains a natural phosphate-binding motif as a starting template, followed by library construction and panning selection, provides a robust approach in generating difficult-to-find antibodies,” said Herren Wu, VP of antibody discovery and protein engineering at the MedImmune LLC unit of AstraZeneca plc. “It is a powerful approach, and one can envision that it can be applied to generating specific antibodies to other post-translational modifications.”

“The study provides a direct example of how structural biology could be used to guide antibody design,” added Sachdev Sidhu, an investigator at the Ontario Institute for Cancer Research and a professor at the University of Toronto.

“The findings should spur additional interest to generate more antibody structures, and the approach could provide insights on the structures that will be needed to generate mAb libraries against various post-translational modifications.”

Although the approach yielded highly specific mAbs to phosphorylated peptides, Andrew Bradbury told SciBX that he now wants to see additional studies to determine whether phospho-specific antibodies generated with this approach will bind to endogenous phosphorylated proteins and without off-target binding to other proteins. Bradbury is a group leader in the biosciences division at the Los Alamos National Laboratory.

He cautioned that just because an antibody binds a peptide fragment of a protein with a post-translational modification does not mean it will bind the full-length, endogenous protein with the same modification.

**Rare mAbs, reduced effort**

The UCSF group’s strategy could bolster the efficiency of generating high-quality, phospho-specific mAbs and possibly mAbs against other post-translational modifications while reducing the time and resources needed.

**“Our method is done entirely *in vitro* and allows us to generate mAbs with high specificity and affinity for phospho-amino acids within a span of two weeks. Traditional hybridoma-based methods take at least two months just to isolate the clones you want.”**

**—James Koerber,  
University of California,  
San Francisco**

“The speed of this approach is substantially faster than others, and the success rate of finding multiple unique antibodies with decent affinity is much higher,”

**“I have no doubt that this approach will generate a high degree of interest in industry and academia as being able to rapidly generate high-quality mAbs specific for post-translational modifications. It can greatly facilitate the study of disease mechanisms and cell biology.”**

—Herren Wu, MedImmune LLC

Wu told *SciBX*. “It would also be relatively easy to adopt this approach in laboratories that have existing capability and capacity in structural biology and combinatorial library generation and screening.”

“Our method is done entirely *in vitro* and allows us to generate mAbs with

high specificity and affinity for phospho–amino acids within a span of two weeks,” said James Koerber, the paper’s lead author and a **Life Sciences Research Foundation** postdoctoral fellow at UCSF. “Traditional hybridoma-based methods take at least two months just to isolate the clones you want. You will then need to do additional screening and scale-up production, which takes additional time and resources.”

“Since we are building recognition for phospho–amino acids into our initial step, we don’t need to carry out the additional screening and counterselection steps required by traditional methods,” added Wells.

Because antibody generation is entirely *in vitro*, the approach is also amenable to automation.

Koerber said that the sequencing and structural analysis steps give the group a better idea of how to design antibodies specific for post-translational modifications, including insights into what types of post-translational modifications could be targeted and which amino acid residues to mutate in the antibody scaffolds.

Sidhu added that the strategy shows that it is possible to bootstrap the way to a desired structural model.

“The group didn’t generate antibody libraries using an existing scaffold but instead used their knowledge of existing antibody structures

and structural modeling to rationally design new scaffolds that they then used to generate their libraries,” he told *SciBX*.

### Broadening horizons

A key next step will be to determine how broadly applicable the strategy is.

“I have no doubt that this approach will generate a high degree of interest in industry and academia as being able to rapidly generate high-quality mAbs specific for post-translational modifications. It can greatly facilitate the study of disease mechanisms and cell biology,” said Wu. “It would be great to see how broadly this approach can be expanded to work on other post-translational modifications, such as acetylation and ubiquitination.”

Koerber said that the group now is trying to automate the approach for generating phospho-specific mAbs.

Wells said that the team is looking into the potential development of mAbs against other post-translational modifications such as acetylation, methylation, sulfation and proteolysis.

UCSF has filed for a provisional patent covering the technology, which is available for licensing.

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# lncRNA meets the androgen receptor

By Chris Cain, Senior Writer

Although many long noncoding RNAs are upregulated in cancer, relatively few have been shown to functionally contribute to disease.<sup>1</sup> Now, a U.S. team has found that two lncRNAs act directly on the androgen receptor and are required for castration-resistant prostate cancer growth. The findings provide new targets for the disease and illustrate a previously unknown mechanism of action for lncRNAs.<sup>2</sup>

Extensive genome profiling studies have identified hundreds of lncRNAs associated with cancer, including prostate cancer.<sup>3-5</sup> Among the best-studied examples is prostate cancer antigen 3 (PCA3), a prostate-specific lncRNA that is overexpressed in patients with prostate cancer.

Based in part on this work, Gen-Probe Inc. developed Prognensa, a prognostic assay that is marketed in the U.S. and Europe to measure levels of PCA3 to determine the need for a repeat biopsy in men suspected of having prostate cancer. Gen-Probe was acquired last year by diagnostics company **Hologic Inc.**

In contrast to these correlative studies, there are few functional studies of prostate cancer-specific lncRNAs or cancer-associated lncRNAs in general.<sup>1</sup> Indeed, even the function of PCA3 remains unclear.

One reason for the lack of functional studies is that the prevalence of lncRNA expression has only been appreciated in the past few years. Another reason is the difficulty of characterizing RNA, which is generally less stable than protein and can be hard to isolate for biochemical analysis.

Now, a team from the **University of California, San Diego** and **The University of Texas MD Anderson Cancer Center** has used a suite of immunoprecipitation techniques and knockdown studies to show how two lncRNAs functionally contribute to prostate cancer growth.

The group honed in on prostate cancer non-coding RNA 1 (PRNCR1), which is encoded in a region of the genome associated with susceptibility to prostate cancer, and prostate-specific transcript 1 (PCGEM1), whose expression had previously been associated with an increased risk of prostate cancer.

RNA immunoprecipitation identified proteins associated with each of these lncRNAs and showed that both associate with the androgen receptor (AR) in prostate cancer tissue. Additional biochemical pull-down and mass spectrometry experiments found multiple proteins associated with the lncRNAs, including histone methyltransferase DOT1L (DOT1L) and the RNA-binding protein pygopus homolog 2 (PYGO2).

To probe the functional role of PRNCR1 and PCGEM1, the team turned to cultured prostate cancer cell lines. In cell lines sensitive to dihydrotestosterone, individual small hairpin RNA against the lncRNAs decreased gene activation by AR compared with control shRNA.

In castration-resistant prostate cancer (CRPC) cell lines, in which the lncRNAs were shown to be overexpressed, knockdown of either lncRNA also decreased gene activation by AR compared with no knockdown.

Thus, PRNCR1 and PCGEM1 are required for both androgen-dependent and androgen-independent activation of target genes by AR.

Additional knockdown and immunoprecipitation experiments led the team to propose a pathway to explain how PRNCR1 and PCGEM1 contribute to AR activity.

First, AR binds PRNCR1 directly at specific acetylated residues. Then DOT1L is recruited, which methylates a specific lysine residue on the receptor. The methylated receptor is then bound by the PCGEM1 lncRNA, which binds and recruits the PYGO2 protein. This complex, including PYGO2, enhances looping of chromatin regions bound by AR and thus promotes the expression of AR target genes.

To firm up the link between these lncRNAs and a functional role in disease, the team tested the relevance of PCGEM1 and PRNCR1 function to prostate cancer growth *in vivo*. To do this, the researchers generated a CRPC cell line with inducible shRNA knockdown of PCGEM1 or PRNCR1 and used it to make xenograft mouse models of prostate cancer.

In this model, knockdown of either lncRNA decreased tumor growth compared with no knockdown.

Results were published in *Nature*. The study was led by Liuqing Yang and Chunru Lin, assistant professors of molecular and cellular oncology at MD Anderson.

The work started while both were postdocs in the lab of Michael Rosenfeld, a corresponding author on the manuscript, a professor of medicine at UCSD and a **Howard Hughes Medical Institute** investigator.

## Expanding the scope

Claes Wahlestedt, associate dean and director of the Center for Therapeutic Innovation and a professor of psychiatry and behavioral sciences at the **University of Miami Miller School of Medicine**, told *SciBX* that the methods used to map this pathway could serve as a model for how to dissect lncRNA function.

“In this paper the authors utilized many innovative and powerful methods to deeply characterize the function of PRNCR1 and PCGEM1. They performed RNA immunoprecipitation, chromatin isolation by RNA purification, protein pull-down using biotinylated RNA, chromatin immunoprecipitation and chromosome conformation capture,” he said. “These techniques represent part of the gold-standard strategy for functionally characterizing lncRNAs and could be applied to study the function of many other disease-associated lncRNAs.”

Howard Chang, a professor of dermatology at the **Stanford University School of Medicine** and an HHMI early career scientist, agreed. “Certainly many other lncRNAs have been found to have altered patterns of expression in many kinds of human cancers and other disease states. They would benefit from functional analyses of this type,” he said.

Chang, whose lab is focused on understanding lncRNA function, wrote a news summary that was published alongside the article in *Nature* and highlighted the work.<sup>6</sup>

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“I believe this study is going to stimulate many other groups to look closely at the lncRNA expressed in various disease states for similar associations that could be therapeutically relevant.”

—Arthur Krieg,  
RaNA Therapeutics Inc.

# Ex-SASP-erating cancer

By Michael J. Haas, Senior Writer

German researchers have identified a hypermetabolic phenotype in senescent tumor cells that exerts tumorigenic effects on other cells in the tumor microenvironment.<sup>1</sup> Although the team showed that small molecule inhibitors of the phenotype reduced tumor growth and improved survival in a mouse model of lymphoma, future studies will need to determine whether the phenotype occurs in primary tumors.

Although tumor cells rendered senescent by chemotherapy are no longer malignant, they can still pose a problem for patients with cancer. Multiple preclinical studies have shown that chemotherapy-treated cancer cells can acquire the senescence-associated secretory phenotype (SASP) to produce inflammatory cytokines, growth factors and proteases. These factors can have tumorigenic effects on other cells in the tumor microenvironment and thereby contribute to disease progression.<sup>2-4</sup>

Moreover, the biology of senescent tumor cells is poorly understood

because of challenges in detecting and separating them from cells actually killed by chemotherapy.

To overcome the challenges in selecting or enriching for such cells to study them, the German team first developed mouse models of lymphoma in which all tumor cells would become senescent—but not die—in response to chemotherapy. They accomplished this by modifying an established transgenic mouse lymphoma cell line to express the antiapoptotic protein B cell lymphoma 2 (BCL-2; BCL2) and then injected the cell line into two groups of mice.

One group of mice was deficient in *suppressor of variegation 3-9 homolog 1* (*Suv39h1*), a histone methyltransferase that plays a role in senescence. These senescence-deficient models and their tumors served as controls.

The other group of mice had normal *Suv39h1* expression. In these models, chemotherapy caused the tumor cells to become senescent instead of dying, thus leaving the cells viable and available for study.

Tumor cells from the chemotherapy-treated, senescence-capable models exhibited hypermetabolic activity that involved increased levels of glucose, glycolysis, proteotoxic stress and autophagy compared with pretreatment baselines and tumor cells from the senescence-deficient controls regardless of treatment status.

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(Continued from “lncRNA meets the androgen receptor,” p. 4)

Arthur Krieg, president, cofounder and CEO of **RaNA Therapeutics Inc.**, told *SciBX*, “I believe this study is going to stimulate many other groups to look closely at the lncRNA expressed in various disease states for similar associations that could be therapeutically relevant.”

RaNA is developing short oligonucleotides that disrupt the ability of lncRNAs to recruit polycomb repressive complex 2 (PRC2) and thus repress target genes.

The key next step for Yang and Lin’s team is to further establish the contribution of PRNCR1 and PCGEM1 in patients with prostate cancer.

Lin said that her team is now developing locked nucleic acids (LNAs) targeting PRNCR1 and PCGEM1 and plans to test the LNAs in additional mouse models of prostate cancer. She also plans to look at the expression and functional contribution of these lncRNAs in a wide range of CRPC samples at various stages of disease progression.

Wahlestedt agreed that the lncRNAs might make for attractive targets but said developing oligonucleotide therapeutics for solid tumors remains a challenge.

“In my opinion, targeting the two lncRNAs may have very promising clinical applications, but the main problem remains how to target lncRNAs. [Small interfering] RNA and antisense oligonucleotides, although very efficacious *in vitro*, have certain limitations when utilized *in vivo*,” he told *SciBX*.

**Enzon Pharmaceuticals Inc.** had an AR-targeting LNA antisense oligonucleotide, EZN-4176, in Phase I trials to treat prostate cancer. Last December, Enzon said that based on the data, it would suspend clinical development of the program to conserve cash. The compound was in development in collaboration with **Santaris Pharma A/S**.

Wahlestedt wanted to see more work done to understand how *PRNCR1* and *PCGEM1* are regulated in prostate cancer cells because

an alternative approach could be to identify small molecules that reduce their expression.

Lin said her team is continuing to study how the lncRNAs are regulated. The findings are unpatented.

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## COMPANIES AND INSTITUTIONS MENTIONED

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**Hologic Inc.** (NASDAQ:HOLX), Bedford, Mass.  
**Howard Hughes Medical Institute**, Chevy Chase, Md.  
**RaNA Therapeutics Inc.**, Cambridge, Mass.  
**Santaris Pharma A/S**, Horsholm, Denmark  
**Stanford University School of Medicine**, Stanford, Calif.  
**University of California, San Diego**, La Jolla, Calif.  
**University of Miami Miller School of Medicine**, Miami, Fla.  
**The University of Texas MD Anderson Cancer Center**, Houston, Texas

In the senescence-capable models pretreated with chemotherapy, small molecule inhibitors of glucose transporters, glycolysis or autophagy decreased tumor growth and increased survival compared with no treatment.

Lastly, the team showed that chemotherapy-induced senescence led to the same hypermetabolic phenotype in primary acute myeloid leukemia (AML) cells and in human lymphoma, leukemia, sarcoma, melanoma, colon cancer, lung cancer and other cancer cell lines. In all of these cell types, the inhibitors of glucose transporters, glycolysis or autophagy decreased tumor cell viability compared with no treatment.

“Our studies with the inhibitors demonstrate the dependence of senescent tumor cells on the hypermetabolic phenotype,” team leader Clemens Schmitt told *SciBX*. “If you cut the energy supply or inhibit autophagy, the cells lose an essential mechanism—the energy-demanding process of autophagy—for coping with senescence-associated proteotoxic stress, and they subsequently die.”

Schmitt is a clinician specializing in hematology and oncology and director of the Molecular Cancer Research Center at **Charité–University Hospital Berlin**. He is also head of the Cancer Genetics and Cellular Stress Responses group at the **Max Delbrueck Center for Molecular Medicine**.

The team included researchers from the **German Cancer Research Center, Jena University Hospital, Johannes Gutenberg University Mainz, the Max Planck Institute of Molecular Plant Physiology, Technical University Munich** and the **University Hospital of Wuerzburg**.

Data were reported in *Nature*.

“This study is very interesting because it provides novel ways to selectively eliminate senescent cells in tissues,” said Francis Rodier, an assistant professor of radiology, radio-oncology and nuclear medicine at the **University of Montreal**.

Previously this was possible only in a few mouse models in which senescent cells—normal or cancerous—were modified to express a protein that could be therapeutically targeted,<sup>5,6</sup> he said.

Rodier also is a researcher at the **Montreal Cancer Institute**.

Masashi Narita, group leader at the **Cancer Research UK Cambridge Institute** at the **University of Cambridge**, agreed that the study provided a new approach for eliminating senescent tumor cells. He said that SASP can trigger an immune system response that eliminates senescent normal cells but that it is not clear how effectively that mechanism clears senescent cancer cells.

“Perturbation of the hypermetabolic state in the senescent tumor cells—which are the source of SASP—kills those cells” and could increase the overall efficiency with which they are eliminated, thereby reducing the potential effects of SASP on disease progression, said Narita.

He added that autophagy was the most attractive process to target therapeutically in the hypermetabolic phenotype. “Chloroquine can inhibit autophagy and has long been used to treat malaria in humans,” which gives it a safety track record, he noted.

Schmitt agreed. “Our results in mice illustrate a therapeutic strategy

that could be used in the clinic to treat patients with lymphomas, leukemias and solid tumors—a sequential approach in which conventional chemotherapy is followed by a metabolic reprogramming agent, such as an autophagy inhibitor” to target the chemotherapy-induced hypermetabolic phenotype of the senescent cells, he said.

### Old cells, new questions

A key question going forward is whether the senescence-related hypermetabolic phenotype actually occurs in human tumors.

Narita said that the team identified and targeted the phenotype in human cells and cell lines but not in xenograft tumor models. Thus, “*in vivo* confirmation of the findings in different tumor types would be necessary,” he said.

He also noted that many human cancers are deficient in p53 and retinoblastoma

protein—tumor suppressor proteins that normal cells need to become senescent. “It is not clear how or whether cells deficient in these proteins can become senescent,” he said. “Thus, I wonder if the team’s approach applies to tumor cells that lack them.”

Rodier said, “A next step is to demonstrate that this metabolic imbalance occurs in human tumors following chemotherapy treatment.”

Schmitt said that his team has not yet conducted studies to evaluate which therapeutic strategy for eliminating senescent tumor cells—inhibition of glucose uptake, glycolysis or autophagy—has the greatest potential for translation to the clinic. However, he said that his team might begin those studies in the near future.

The findings reported in the paper are unpatented, and the team is open to corporate partnerships to develop them, Schmitt said.

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### COMPANIES AND INSTITUTIONS MENTIONED

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**Max Planck Institute of Molecular Plant Physiology**, Potsdam, Germany  
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**“A next step is to demonstrate that this metabolic imbalance occurs in human tumors following chemotherapy treatment.”**

**—Francis Rodier,  
University of Montreal**

# Epilepsy narrows down

By Lev Osherovich, Senior Writer

A spate of human genetic studies has shed light on the root causes of idiopathic focal epilepsy, a common childhood form of the disease. The studies suggest that a group of epilepsies and encephalopathies marked by seizures and language and learning disorders can result from mutations in NMDAR subunits.

Because some of the epilepsy-associated mutations appear to cause excessive NMDAR activity, the findings build a case for testing receptor subunit-selective antagonists to treat idiopathic focal epilepsy and possibly other forms of epilepsy.

At least two companies developing subunit-selective NMDA modulators—**Naurex Inc.** and **Mnemosyne Pharmaceuticals Inc.**—see childhood epilepsy as a potential new area of focus if basic mechanistic questions as well as a clinical testing strategy can be worked out.

NMDARs are a family of ion channels used by neurons to relay signals in response to glutamate, an excitatory neurotransmitter.

In the 1980s and 1990s, nonselective NMDAR channel blockers such as ketamine were tested in a range of neurology indications including epilepsy but encountered safety problems including dissociation and psychosis.

“NMDARs have been of interest in epilepsy since the 1980s, but at that time we didn’t have selective compounds,” said Henrik Klitgaard, VP of CNS research at **UCB Group**. “The first NMDA nonselective antagonist tested for epilepsy had very severe psychiatric effects, so that approach was abandoned.”

Today, the modestly potent NMDAR antagonist Namenda memantine is marketed for Alzheimer’s disease (AD) by **Forest Laboratories Inc.** and **Merz GmbH & Co. KGaA**.

Now, findings from three independent international teams<sup>1-3</sup> may rekindle interest in testing newer, more selective NMDA modulators in childhood epilepsy.

The studies show that up to 20% of idiopathic focal epilepsy cases arise from mutations in an NMDAR subunit called *NMDA receptor NR2A subtype* (*GRIN2A*; *NR2A*).

## Search and seizure

The teams converged on *NR2A* by studying families with a history of idiopathic focal epilepsy, a broad set of disorders including rolandic epilepsy, atypical benign partial epilepsy, Landau-Kleffner syndrome (LKS) and epileptic encephalopathy with continuous spike and waves during slow-wave sleep (CSWSS).

“Electroencephalographic abnormalities are the hallmark of idiopathic focal epilepsy, which begins in childhood,” said Sarah von Spiczak, a professor of pediatrics at **Kiel University**. “This is about 15%–20% of pediatric epilepsy cases.”

Spiczak and coleader Holger Lerche, a professor of neurology at

the **University of Tuebingen**, previously identified three patients with idiopathic focal epilepsy who had deletions encompassing *NR2A*.<sup>4</sup>

In their new study, the duo found point mutations or microdeletions in *NR2A* in 30 of 259 patients. Those with *NR2A* mutations typically had more severe forms of idiopathic focal epilepsy than patients with intact *NR2A*.

Independently, a team led by Pierre Szepetowski, research director at the **Centre National de la Recherche Scientifique** (CNRS) and the Mediterranean Institute of Neurobiology (INMED) at **Aix-Marseille University**, identified *NR2A* mutations as the likely cause of about 20% of cases among a panel of 66 patients with LKS or CSWSS.

Finally, a team led by **University of Washington** assistant professor of pediatrics Heather Mefford and **The University of Melbourne** professor of medicine and pediatrics Ingrid Scheffer sequenced *NR2A* in 519 pediatric patients with a broad range of epilepsy and language disorders. Among the 44 patients who had the electroencephalographic abnormalities and speech disorders that are the hallmarks of idiopathic focal epilepsy, 4 had mutations in *NR2A*.

No patients in the cohort with other forms of epilepsy had *NR2A* mutations.

Altogether, the three studies suggest that *NR2A* mutations are the most common individual genetic cause of idiopathic focal epilepsy. *NR2A* mutations cause “between 7% and 20% of cases and are the only known genetic cause to date,” said Szepetowski.

What remains unclear is what role NMDARs play in the remaining 80% of idiopathic focal epilepsy cases not attributed to *NR2A* mutations.

To find out, Mefford is doing more genetic studies in the hopes of identifying further risk factors of idiopathic focal epilepsy and other pediatric epilepsies. “We’re still focusing on gene discovery, continuing the targeted candidate gene approach with a larger set of patients and parents,” said Mefford.

Szepetowski is not convinced that more genes with as strong an effect as *NR2A* will turn up. “We don’t know the extent of heterogeneity in the remaining 80% of cases,” he said. “We don’t even know if the other cases are even genetic.”

He noted that idiopathic focal epilepsy previously was thought to involve autoimmune activity against NMDARs, but there was no evidence of autoimmunity in any of the patients in Szepetowski’s cohort. However, it is still possible that autoimmunity plays a role in the remaining cases.

The findings of all three teams were reported in *Nature Genetics*.

## Function disjunction

From a genetic standpoint, the disease-linked mutations are likely to lead to loss of function of *NR2A* protein. However, NMDARs are highly complex, with multiple points of interaction between subunits and multiple binding sites for glutamate, glycine and zinc, which fine-tune the receptors’ activities.

Thus, it is hard to predict how epilepsy-associated mutations would affect NMDAR activity.

The teams’ leaders were divided about whether NMDARs could make good drug targets but agreed that further mechanistic details

“The pharmacopeia right now is very limited, but these studies usher in a new dawn of specific, targeted therapy.”

—Joseph Moskal,  
Northwestern University

about how the mutations lead to disease could paint a clearer picture about how to proceed with drug development.

“These studies argue for targeting NMDARs” in idiopathic focal epilepsy, said Mefford.

But Lerche and Spiczak cautioned that the functional consequences of the mutations, and thus the appropriate therapeutic strategy, are not well understood.

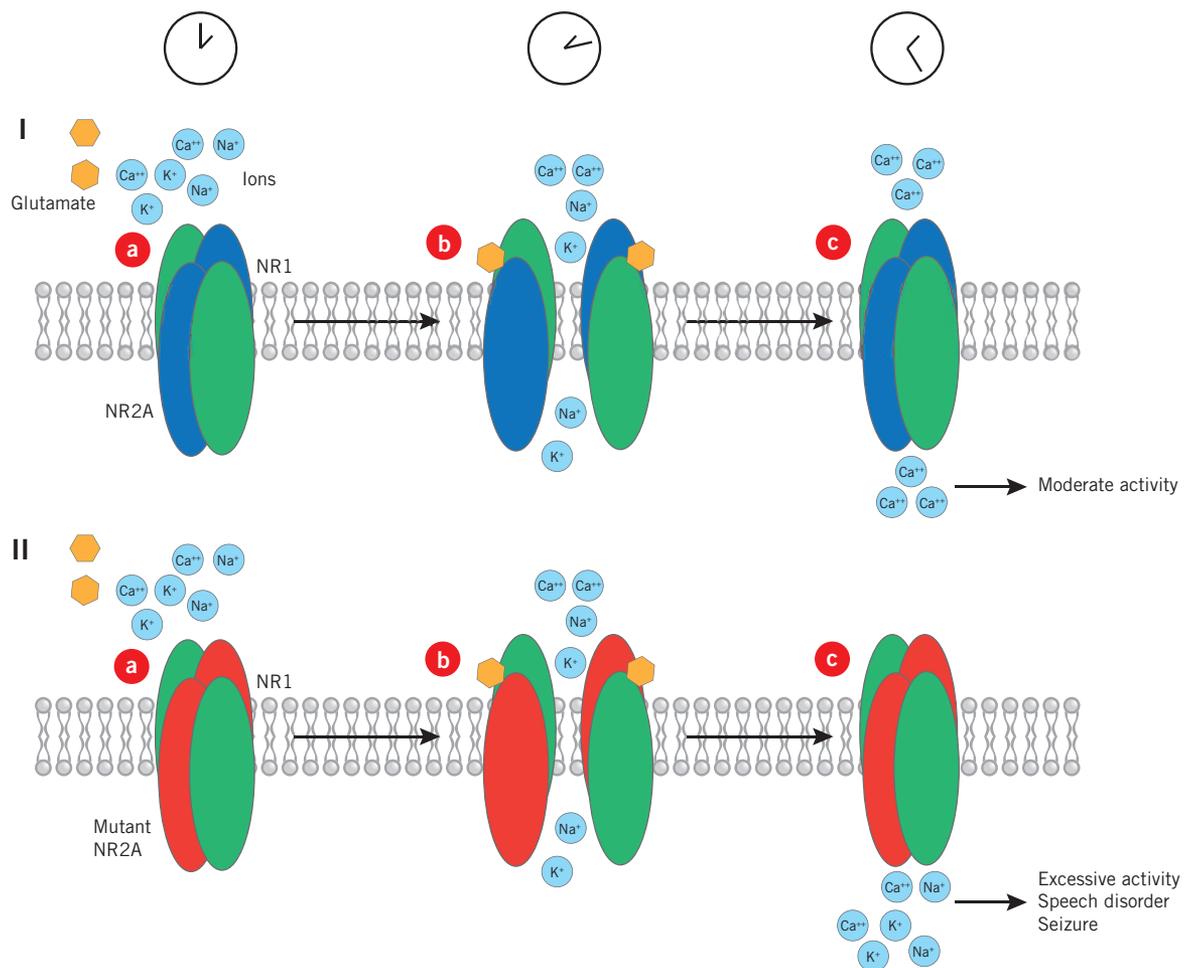
“The most severe mutations are total disruptions, but some of the point mutations are not entirely clear and [are] perhaps a gain of function,” said Lerche.

Added Spiczak, “We don’t have enough information to answer conclusively whether NR2A is a good target.”

Szpetowski said that ongoing cell culture and animal studies of how the disease-linked mutations affect NMDAR activity will provide a better picture of how to treat idiopathic focal epilepsy, which may require individually tailored approaches for each type of *NR2A* mutation.

Each team took preliminary steps to characterize the functional consequences of some of the disease-associated *NR2A* mutations on NMDAR activity in cell culture.

The Spiczak and Lerche team found that one disease-linked mutation



**Figure 1. NMDARs in epilepsy.** Reports by three independent groups suggest mutations in *NMDA receptor NR2A subtype (GRIN2A; NR2A)* underlie a subset of epilepsy-aphasia spectrum disorders.

The groups found *de novo* mutations in *NR2A* in 9%–20% of patients displaying a range of epileptic disorders characterized by abnormal slow wave activity, seizure and language deficits. Studies in cell culture suggest the disease-associated mutations lead to abnormal NMDAR kinetics.

In early childhood, NMDARs are heterotetrameric ion channels (I[a]) consisting of two subunits of NMDA receptor NR1 subtype (GRIN1; NR1) and two subunits of NR2A. Normally, binding of the excitatory neurotransmitter glutamate leads to opening of the channel, allowing influx of ions into neurons (I[b]). After a short time, the channel closes, leading to moderate accumulation of ions in the cytoplasm and normal neurological activity (I[c]).

In some patients with epilepsy-aphasia spectrum disorders, mutations in *NR2A* (II[a]) compromise the kinetics of NMDAR activity. Glutamate binding to mutant *NR2A*-containing NMDARs (II[b]) leads to a prolonged open state (II[c]) and excessive ion influx, which in turn causes overactivation of downstream intracellular signaling, speech disorders and seizures.

from among their cohort reduced the binding of zinc, which inhibits NMDAR activity. As a result, the mutant receptor responded normally to glutamate but could not be turned off by zinc.

Szepetowski's team tested a different disease-linked *NR2A* mutant in cell culture and saw a delay in the closing of the NMDAR's ion channel after a period of activation. As a result, the mutant receptor had a longer window of activity than the wild-type receptor control.

Mefford and Scheffer's team characterized a third *NR2A* mutation in cell culture and, like Szepetowski's team, saw a delay in channel closing.

Altogether, the functional analysis of disease-linked *NR2A* mutations suggests that in at least some patients with idiopathic epilepsy, NMDAR channels remain open or active for too long, allowing the influx of an excessive number of ions (see Figure 1, "NMDARs in epilepsy").

### Loose fit

The new findings raise the possibility of repurposing existing NMDAR modulators for epilepsy. At least 20 NMDAR antagonists are marketed or in development for a broad range of neurology indications, but none are in development for epilepsy.

Indeed, the standard of care for most forms of epilepsy is anticonvulsant drugs that are thought to act downstream of the disorder's primary causes, which have been poorly understood until now.

"The pharmacopeia right now is very limited, but these studies usher in a new dawn of specific, targeted therapy" for epilepsy, said Joseph Moskal, a professor of biomedical engineering and director of the Falk Center for Molecular Therapeutics at **Northwestern University**. "The ideal pharmacological agent would dampen down the protein. You want a gentle dampener, not a robust antagonist. An awful lot of NR2A is needed elsewhere in the central nervous system."

Moskal is founder and CSO of Naurex, which is developing NMDAR modulators for pain and depression. The company's lead product is GLYX-13, a partial agonist of the NMDAR's glycine-binding site. GLYX-13 is in Phase II testing for depression.

For now, Moskal said that further physiological characterization of epilepsy-associated mutations in cell culture and animal models would help work out exactly how to target NMDARs.

"I'd like to see these mutations expressed in transgenic mice to study hippocampal slices and brain physiology," said Moskal.

Frank Menniti, cofounder and CSO of Mnemosyne, said that targeting NR2A-bearing NMDARs could yield better results than the high-potency, nonselective antagonists of the past.

"NMDARs are so broadly involved in brain function and development that it's in some way surprising that there are specific effects of these mutations" that cause disease rather than outright failure of brain development, said Menniti.

Mnemosyne has discovery stage programs for positive and negative allosteric modulators of NMDARs for schizophrenia, depression and other neurological disorders.

UCB's Klitgaard said NR2A has not been as thoroughly explored as other NMDAR components such as NR2B (GRIN2B).

"There are NR2B-selective compounds, but I don't know if NR2A-selective compounds exist," said Klitgaard. "They may exist on the shelves

**"There are NR2B-selective compounds, but I don't know if NR2A-selective compounds exist. They may exist on the shelves of pharma."**

—Henrik Klitgaard, UCB Group

of pharma."

Industry's challenge is to find NR2A-selective compounds that correct the abnormal activity of the mutated protein without perturbing NMDAR signaling throughout the brain.

"The glutamatergic system, especially NMDARs, is among the most complex neurotransmitter systems in the brain," said

Szepetowski. "We would not want to just turn down this system because this might have very adverse effects."

An open question is whether modulating NR2A or other NMDAR subunits will be effective in other forms of epilepsy besides idiopathic focal epilepsy.

"My guess is that NR2A antagonists would have a much broader profile of antiepileptic activity, including adult forms, for which there has not yet been any genetic association with the NMDAR," said Menniti.

Another difficulty lies in clinical trial design in which palliative anticonvulsants are the standard of care.

"From a big pharma drug development standpoint, epilepsy has been a scary thing to get involved in," said Menniti. "If you have someone whose seizures are under control with an existing drug, you can't pull them off the drug to try your new compound."

None of the findings reported in the three papers have been patented.

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### COMPANIES AND INSTITUTIONS MENTIONED

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**Centre National de la Recherche Scientifique**, Marseille, France  
**Forest Laboratories Inc.** (NYSE:FRX), New York, N.Y.  
**Kiel University**, Kiel, Germany  
**Merz GmbH & Co. KGaA**, Frankfurt, Germany  
**Mnemosyne Pharmaceuticals Inc.**, Providence, R.I.  
**Naurex Inc.**, Evanston, Ill.  
**Northwestern University**, Evanston, Ill.  
**UCB Group** (Euronext:UCB), Brussels, Belgium  
**The University of Melbourne**, Melbourne, Victoria, Australia  
**University of Tuebingen**, Tuebingen, Germany  
**University of Washington**, Seattle, Wash.

## 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
<b>Cancer</b>				
Brain cancer	Epidermal growth factor receptor (EGFR); Janus kinase-2 (JAK-2); PTEN (MMAC1; TEP1)	<i>In vitro</i> and mouse studies suggest allosteric inhibition of JAK-2 could help treat glioblastoma multiforme (GBM). In <i>PTEN</i> -deficient glioblastoma cell lines with constitutively active EGFR, compared with normal neural progenitor cells and astrocytes, the small molecule G5-7 selectively decreased proliferation. <i>In vitro</i> assays revealed that G5-7 bound to and allosterically inhibited JAK-2. In subcutaneous and intracranial xenograft mouse models of GBM, G5-7 decreased tumor growth compared with vehicle. Next steps include elucidating G5-7's mechanism of action and optimizing the compound for clinical development. Incyte Corp. and Novartis AG market Jakafi ruxolitinib, a JAK-1 and JAK-2 inhibitor, to treat myeloproliferative disorder. At least nine companies have JAK-2 inhibitors in Phase III testing or earlier development for various indications.  <b>SciBX 6(34); doi:10.1038/scibx.2013.917</b> <b>Published online Sept. 5, 2013</b>	Patented by Emory University; licensed to Advinus Therapeutics Ltd.	He, K. <i>et al. Sci. Signal.</i> ; published online July 9, 2013; doi:10.1126/scisignal.2003900 <b>Contact:</b> Keqiang Ye, Emory University School of Medicine, Atlanta, Ga. e-mail: <a href="mailto:kye@emory.edu">kye@emory.edu</a>
Breast cancer	Src; epidermal growth factor receptor 1 (EGFR1; HER1; ErbB1); HER2 (EGFR2; ErbB2; neu)	Human tumor and mouse studies suggest Src inhibitors could help treat brain metastases from breast cancer. In patients who have <i>HER2</i> <sup>+</sup> , metastatic breast cancer and mouse models of <i>HER2</i> <sup>+</sup> or triple-negative breast cancer, Src expression and activation levels were higher in brain metastases than those in the primary tumors. In mice xenograft models with <i>HER2</i> <sup>+</sup> or triple-negative brain metastases, Tykerb lapatinib plus a Src inhibitor decreased tumor growth and increased survival compared with either agent alone. Ongoing work includes confirming the findings in the mouse models using the Src inhibitor Sprycel dasatinib. GlaxoSmithKline plc markets Tykerb, a HER1 and HER2 kinase inhibitor, to treat breast cancer. Bristol-Myers Squibb Co. and Otsuka Pharmaceutical Co. Ltd. market Sprycel, a small molecule inhibitor of BCR-ABL tyrosine kinase and Src, to treat acute lymphoblastic leukemia (ALL) and chronic myelogenous leukemia (CML). The partners also have the drug in Phase II testing to treat breast and pancreatic cancers and Phase I testing to treat leukemia. Pfizer Inc.'s Bosulif bosutinib, a dual inhibitor of BCR-ABL and Src, is marketed to treat CML and in Phase II testing to treat polycystic kidney disease (PKD). Kinex Pharmaceuticals LLC and Hanmi Pharmaceutical Co. Ltd. have KX01 (KX2-391), a selective, small molecule, non-ATP Src inhibitor, in Phase II testing or earlier to treat various cancers.  <b>SciBX 6(34); doi:10.1038/scibx.2013.918</b> <b>Published online Sept. 5, 2013</b>	Unpatented; available for partnering	Zhang, S. <i>et al. Cancer Res.</i> ; published online Aug. 1, 2013; doi:10.1158/0008-5472.CAN-12-1803 <b>Contact:</b> Dihua Yu, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: <a href="mailto:dyu@mdanderson.org">dyu@mdanderson.org</a>
Cancer	c-Myc (MYC); mammalian target of rapamycin (mTOR; FRAP; RAFT1); 3-phosphoinositide dependent protein kinase-1 (PDPK1); polo-like kinase 1 (PLK1; STPK13)	Cell culture and mouse studies suggest combining PLK1 and mTOR inhibitors could help treat MYC-driven cancers. In cultured cells, PLK1 coimmunoprecipitated and phosphorylated MYC in a PDPK1-dependent manner. In a mouse xenograft model of colorectal cancer, the mTOR inhibitor BEZ235 plus a PLK1 inhibitor decreased MYC expression and tumor growth compared with vehicle or either inhibitor alone. Next steps could include combining PLK1 inhibitors with mTOR and PI3K inhibitors. BEZ235, a dual inhibitor of PI3K and mTOR from Novartis AG, is in Phase I/II testing to treat cancer. At least nine companies have PLK1 inhibitors in Phase III testing or earlier to treat cancer.  <b>SciBX 6(34); doi:10.1038/scibx.2013.919</b> <b>Published online Sept. 5, 2013</b>	Patent application filed; available for licensing	Tan, J. <i>et al. Cancer Discov.</i> ; published online July 25, 2013; doi:10.1158/2159-8290.CD-12-0595 <b>Contact:</b> Qiang Yu, Genome Institute of Singapore, Singapore e-mail: <a href="mailto:yuq@gis.a-star.edu.sg">yuq@gis.a-star.edu.sg</a>

## This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer	Epidermal growth factor receptor (EGFR); heat shock protein 90 (Hsp90)	<i>In vitro</i> studies identified a peptide fragment from EGFR that could help treat cancer. EGFR is stabilized by Hsp90 through binding at an eight-amino-acid segment on the receptor. In EGFR-dependent head and neck cancer cells, an EGFR peptide fragment that competes with EGFR binding to Hsp90 caused specific degradation of EGFR. In head and neck cancer cells and non-small cell lung cancer (NSCLC) cells, the peptide inhibited a dimerization process required for EGFR activation and decreased cancer cell survival compared with a scrambled control peptide. Next steps could include testing the peptide in animal models of cancer. Synta Pharmaceuticals Corp.'s Hsp90 inhibitor, ganetespib, is in Phase III testing to treat NSCLC. At least 13 other companies have Hsp90 inhibitors in Phase II testing or earlier to treat various cancers.  <b>SciBX 6(34); doi:10.1038/scibx.2013.920</b> <b>Published online Sept. 5, 2013</b>	Patent and licensing status unavailable	Ahsan, A. <i>et al. J. Biol. Chem.</i> ; published online July 29, 2013; doi:10.1074/jbc.M113.492280 <b>Contact:</b> Mukesh K. Nyati, The University of Michigan Medical School, Ann Arbor, Mich. e-mail: <a href="mailto:nyati@umich.edu">nyati@umich.edu</a>
Cancer	Not applicable	Cell culture and mouse studies suggest inhibiting glucose transporters, glycolysis or autophagy could help eliminate senescent cancer cells. In multiple cancer cell cultures and in a mouse model of lymphoma, chemotherapy-induced senescence increased levels of glucose, glycolysis, proteotoxic stress and autophagy compared with those in cells that were not senescent. In chemotherapy-pretreated cancer cell cultures and mouse models of lymphoma, inhibitors of glucose transporters, glycolysis or autophagy decreased cell viability and tumor growth compared with no treatment. Future studies could include testing additional inhibitors of autophagy in chemotherapy-treated animal models of cancer ( <i>see Ex-SASP-erating cancer, page 5</i> ).  <b>SciBX 6(34); doi:10.1038/scibx.2013.921</b> <b>Published online Sept. 5, 2013</b>	Unpatented; unlicensed; available for partnering	Dörr, J.R. <i>et al. Nature</i> ; published online Aug. 14, 2013; doi:10.1038/nature12437 <b>Contact:</b> Clemens A. Schmitt, Charité–University Hospital Berlin, Berlin, Germany e-mail: <a href="mailto:clemens.schmitt@charite.de">clemens.schmitt@charite.de</a>
Cancer	R-spondin 1 (RSPO1); slit homolog 2 (SLIT2)	Mouse studies suggest RSPO1 and SLIT2 could improve the tolerability of chemoradiotherapy, which is known to damage normal intestinal tissues. In the small intestine of mice, human SLIT2 overexpression increased intestinal stem cell numbers and intestinal regeneration compared with no overexpression. In mice with intestinal tumors that received a lethal dose of chemoradiation, recombinant human SLIT2 and RSPO1 synergistically increased survival compared with either recombinant protein alone. In these mice, SLIT2 and RSPO1 did not dampen the efficacy of chemoradiation. Next steps include generating longer-lived chimeric proteins by fusing human SLIT2 and RSPO1 to immunoglobulin Fc fragments.  <b>SciBX 6(34); doi:10.1038/scibx.2013.922</b> <b>Published online Sept. 5, 2013</b>	Patent application filed; available for licensing	Zhou, W.-J. <i>et al. Nature</i> ; published online July 31, 2013; doi:10.1038/nature12416 <b>Contact:</b> Jian-Guo Geng, University of Michigan School of Medicine, Ann Arbor, Mich. e-mail: <a href="mailto:jjgeng@umich.edu">jjgeng@umich.edu</a>
Cancer	S-phase kinase-associated protein 2 (SKP2)	<i>In vitro</i> , cell culture and mouse studies identified SKP2 inhibitors that could be useful for treating cancer. An <i>in silico</i> screen and <i>in vitro</i> assays identified a small molecule, 3-(1,3-benzothiazol-2-yl)-6-ethyl-7-hydroxy-8-(1-piperidinylmethyl)-4H-chromen-4-one, that blocked the interaction between SKP1 and SKP2 at low micromolar concentrations and specifically inhibited the ubiquitination of SKP2 substrates. In cancer cell lines, the compound decreased growth and the expression of cancer stem cell surface markers compared with vehicle. In mouse xenograft models of lung or prostate cancer, the compound also decreased tumor growth. Next steps include lead optimization.  <b>SciBX 6(34); doi:10.1038/scibx.2013.923</b> <b>Published online Sept. 5, 2013</b>	Patent application filed; available for licensing	Chan, C.-H. <i>et al. Cell</i> ; published online Aug. 1, 2013; doi:10.1016/j.cell.2013.06.048 <b>Contact:</b> Hui-Kuan Lin, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: <a href="mailto:hklin@mdanderson.org">hklin@mdanderson.org</a> <b>Contact:</b> Shuxing Zhang, same affiliation as above e-mail: <a href="mailto:shuzhang@mdanderson.org">shuzhang@mdanderson.org</a>

## This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer	VEGF receptor 1 (FLT1; VEGFR-1)	<i>In vitro</i> and mouse studies suggest increasing monocyte production of soluble FLT1 could enhance the effects of therapeutic antibodies against cancer. In cultured peripheral blood monocytes, stimulation and activation with IgG or therapeutic antibodies increased soluble FLT1 levels compared with no stimulation. In human umbilical vein endothelial cells, supernatant from stimulated monocytes containing soluble FLT1 inhibited angiogenesis compared with supernatant from unstimulated cells. In a mouse model of cancer, a neutralizing antibody targeting soluble FLT1 weakened the anticancer effect of a therapeutic antibody. Next steps could include evaluating the combination of soluble FLT1 and a therapeutic mAb in animal cancer models.  <b>SciBX 6(34); doi:10.1038/scibx.2013.924</b> <b>Published online Sept. 5, 2013</b>	Patent and licensing status unavailable	Justiniano, S.E. <i>et al. J. Biol. Chem.</i> ; published online July 31, 2013; doi:10.1074/jbc.M113.485185 <b>Contact:</b> Susheela Tridandapani, The Ohio State University, Columbus, Ohio e-mail: <a href="mailto:tridandapani.2@osu.edu">tridandapani.2@osu.edu</a>
Cervical cancer	Histone deacetylase 10 (HDAC10)	Patient tissue and mouse studies suggest increasing HDAC10 activity could help prevent cervical cancer metastasis. In an analysis of clinical and microarray data in 60 cervical cancer samples, decreased HDAC10 protein levels correlated with increased lymph node metastasis and more advanced disease. In a mouse xenograft model of human cervical cancer, HDAC10 overexpression decreased metastasis compared with normal HDAC10 expression, though it did not inhibit tumor growth. Next steps include determining whether the antimetastatic effects of HDAC10 apply to other cancers.  <b>SciBX 6(34); doi:10.1038/scibx.2013.925</b> <b>Published online Sept. 5, 2013</b>	Unpatented; licensing status not applicable	Song, C. <i>et al. J. Biol. Chem.</i> ; published online July 29, 2013; doi:10.1074/jbc.M113.498758 <b>Contact:</b> Jiuhong Kang, Tongji University, Shanghai, China e-mail: <a href="mailto:jhkang@tongji.edu.cn">jhkang@tongji.edu.cn</a> <b>Contact:</b> Chuanyue Wu, University of Pittsburgh, Pittsburgh, Pa. e-mail: <a href="mailto:carywu@pitt.edu">carywu@pitt.edu</a>
Prostate cancer	Prostate cancer non-coding RNA 1 (PRNCR1); prostate-specific transcript 1 (PCGEM1)	<i>In vitro</i> , cell culture and mouse studies suggest inhibiting the long noncoding RNAs (lncRNAs) PRNCR1 or PCGEM1 could help treat prostate cancer. Chromatin isolation by RNA purification and <i>in vitro</i> binding studies showed that the lncRNAs PRNCR1 and PCGEM1 bound directly to androgen receptor (AR) and associated with AR-regulated genes in a prostate cancer cell line. In this cell line, small hairpin RNA knockdown of either lncRNA decreased AR signaling compared with no knockdown. In a mouse xenograft model of prostate cancer, shRNA against either lncRNA decreased tumor growth compared with control shRNA. Next steps include additional studies on the expression of PRNCR1 and PCGEM1 in prostate cancer patient samples ( <i>see lncRNA meets the androgen receptor, page 4</i> ).  <b>SciBX 6(34); doi:10.1038/scibx.2013.926</b> <b>Published online Sept. 5, 2013</b>	Unpatented; licensing status not applicable	Yang, L. <i>et al. Nature</i> ; published online Aug. 14, 2013; doi:10.1038/nature12451 <b>Contact:</b> Michael G. Rosenfeld, University of California, San Diego, La Jolla, Calif. e-mail: <a href="mailto:mrosenfeld@ucsd.edu">mrosenfeld@ucsd.edu</a> <b>Contact:</b> Chunru Lin, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: <a href="mailto:clin2@mdanderson.org">clin2@mdanderson.org</a> <b>Contact:</b> Liuqing Yang, same affiliation as above e-mail: <a href="mailto:lyang7@mdanderson.org">lyang7@mdanderson.org</a>
Sarcoma	Procollagen-lysine 2-oxoglutarate 5-dioxygenase 2 (PLOD2); hypoxia- inducible factor 1 $\alpha$ (HIF1A; HIF1 $\alpha$ )	Studies in human samples and mice suggest inhibiting PLOD2 could help prevent metastasis in patients with sarcoma. In the tumor microenvironment, PLOD2 is upregulated by HIF1A. In mouse models of sarcoma, small hairpin RNA against <i>Hif1a</i> or <i>Plod2</i> or pharmacological inhibition of <i>Plod2</i> signaling all decreased pulmonary metastasis compared with scrambled shRNA or no inhibition. In human sarcoma samples, HIF1A and PLOD2 expression were greater in metastatic tumors than nonmetastatic tumors. Next steps could include testing PLOD2 inhibition in additional animal models of sarcoma.  <b>SciBX 6(34); doi:10.1038/scibx.2013.927</b> <b>Published online Sept. 5, 2013</b>	Patent and licensing status unavailable	Eisinger-Mathason, T.S.K. <i>et al. Cancer Discov</i> ; published online Aug. 1, 2013; doi:10.1158/2159-8290.CD-13-0118 <b>Contact:</b> M. Celeste Simon, University of Pennsylvania, Philadelphia, Pa. e-mail: <a href="mailto:celeste2@mail.med.upenn.edu">celeste2@mail.med.upenn.edu</a>

## This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
<b>Cardiovascular disease</b>				
Heart failure	BET bromodomain	<p>Cell culture and rodent studies suggest BET bromodomain inhibitors could help treat heart failure. In rodent and human heart tissue, the BET bromodomain-containing protein bromodomain containing 4 (BRD4) was strongly expressed. In neonatal rat ventricular cardiomyocytes, a series of BET bromodomain inhibitors decreased hypertrophy compared with vehicle control. In the transverse aortic constriction mouse model of cardiac hypertrophy, a BET bromodomain inhibitor improved cardiac function and decreased histological features of heart failure compared with vehicle. Next steps could include testing BET bromodomain inhibition in additional animal models. Tensha Therapeutics Inc., cofounded by James Bradner, has the BET bromodomain inhibitor TEN-010 in preclinical development for cancer.</p> <p>At least three other companies have BET bromodomain inhibitors in Phase I testing or preclinical development to treat cancer.</p> <p><b>SciBX 6(34); doi:10.1038/scibx.2013.928</b> Published online Sept. 5, 2013</p>	Patent and licensing status unavailable	<p>Anand, P. <i>et al. Cell</i>; published online Aug. 1, 2013; doi:10.1016/j.cell.2013.07.013 <b>Contact:</b> Saptarsi M. Haldar, Case Western Reserve University School of Medicine, Cleveland, Ohio e-mail: <a href="mailto:saptarsi.haldar@case.edu">saptarsi.haldar@case.edu</a> <b>Contact:</b> James E. Bradner, Dana-Farber Cancer Institute, Boston, Mass. e-mail: <a href="mailto:james_bradner@dfci.harvard.edu">james_bradner@dfci.harvard.edu</a></p>
<b>Infectious disease</b>				
HIV/AIDS	CD4; HIV gp120	<p><i>In vitro</i> studies suggest bispecific antibodies targeting CD4 and gp120 could be useful for treating HIV infection. In a viral neutralization assay, bispecific mAbs engineered to recognize both CD4 and gp120 had better potency than the CD4-specific parent mAb ibalizumab. Next steps include preclinical development of the bispecific antibodies as passive immunotherapy for HIV.</p> <p>In 2007, TaiMed Biologics Inc. licensed ibalizumab from Genentech Inc., now a unit of Roche.</p> <p><b>SciBX 6(34); doi:10.1038/scibx.2013.929</b> Published online Sept. 5, 2013</p>	Patent pending; licensed to TaiMed Biologics	<p>Pace, C.S. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online July 22, 2013; doi:10.1073/pnas.1304985110 <b>Contact:</b> David D. Ho, The Rockefeller University, New York, N.Y. e-mail: <a href="mailto:dho@adarc.org">dho@adarc.org</a></p>
<b>Neurology</b>				
Alzheimer's disease (AD)	$\beta$ -Amyloid (A $\beta$ ); $\beta$ -site APP-cleaving enzyme 1 (BACE1); mammalian target of rapamycin (mTOR); FRAP; RAFT1)	<p>Mouse and cell culture studies suggest the plant-derived compound arctigenin could help treat AD. In a series of human cell lines, arctigenin decreased A<math>\beta</math> production compared with vehicle by inhibiting BACE1 translation. In these cell lines, arctigenin inhibited mTOR signaling and also increased A<math>\beta</math> clearance compared with vehicle. In a transgenic mouse model of AD, arctigenin decreased memory impairment and plaque formation in the brain compared with vehicle. Next steps include seeking an industry collaborator to help develop arctigenin to treat AD.</p> <p><b>SciBX 6(34); doi:10.1038/scibx.2013.930</b> Published online Sept. 5, 2013</p>	Patent pending covering arctigenin and its analogs; licensing status undisclosed	<p>Zhu, Z. <i>et al. J. Neurosci.</i>; published online Aug. 7, 2013; doi:10.1523/JNEUROSCI.4790-12.2013 <b>Contact:</b> Xu Shen, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China e-mail: <a href="mailto:xshen@mail.shcnc.ac.cn">xshen@mail.shcnc.ac.cn</a> <b>Contact:</b> Hualiang Jiang, same affiliation as above e-mail: <a href="mailto:hjjiang@mail.shcnc.ac.cn">hjjiang@mail.shcnc.ac.cn</a> <b>Contact:</b> Lihong Hu, same affiliation as above e-mail: <a href="mailto:simmhulh@mail.shcnc.ac.cn">simmhulh@mail.shcnc.ac.cn</a></p>
Epilepsy	NMDAR	<p>Rat studies suggest D-serine could help treat epilepsy. In a rat model of pilocarpine-induced temporal lobe epilepsy, D-serine levels in the hippocampus were lower than those in nonepileptic rats. In hippocampal brain slices from the epileptic rats, long-term potentiation was impaired. In the rat epilepsy model, dietary supplementation with D-serine decreased deficits in both spatial learning and long-term potentiation compared with no supplementation. Next steps could include testing the effects of D-serine in additional animal models of epilepsy.</p> <p><b>SciBX 6(34); doi:10.1038/scibx.2013.931</b> Published online Sept. 5, 2013</p>	Patent and licensing status unavailable	<p>Klatte, K. <i>et al. J. Neurosci.</i>; published online Aug. 7, 2013; doi:10.1523/JNEUROSCI.5423-12.2013 <b>Contact:</b> Heinz Beck, University of Bonn, Bonn, Germany e-mail: <a href="mailto:heinz.beck@ukb.uni-bonn.de">heinz.beck@ukb.uni-bonn.de</a></p>

## This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Neurology	Adenosine monophosphate deaminase 2 (AMPD2)	<i>In vitro</i> and genetic studies identified a subset of patients with pontocerebellar hypoplasia (PCH) who could benefit from treatment with purine precursors. A genetic screen of 30 patients with early onset PCH and their families identified a subset in the cohort with mutations in <i>AMPD2</i> , an enzyme necessary for biosynthesis of the purine nucleotide guanine. In patient neural progenitor cells, a feedback loop regulating purine biosynthesis was disrupted and treatment with purine precursors rescued protein translation and cell viability. Next steps could include developing animal models of PCH based on mutations in <i>Ampd2</i> .	Patent and licensing status unavailable	Akizu, N. <i>et al. Cell</i> ; published online Aug. 1, 2013; doi:10.1016/j.cell.2013.07.005 <b>Contact:</b> Joseph G. Gleeson, University of California, San Diego, La Jolla, Calif. e-mail: <a href="mailto:jogleeson@ucsd.edu">jogleeson@ucsd.edu</a>
		<b>SciBX 6(34); doi:10.1038/scibx.2013.932</b> Published online Sept. 5, 2013		
Pain	Parathyroid hormone 2 (PTH2; TIP39); PTH2 receptor (PTH2R)	Mouse studies suggest antagonizing PTH2R could help treat chronic pain. In mouse models of chronic neuropathic and inflammatory pain, knockout of the neuropeptide <i>Pth2</i> or its receptor, <i>Pth2r</i> , decreased hypersensitivity reactions compared with no knockout. In mice, lentivirus-mediated expression of a Pth2r antagonist in the locus coeruleus of the brain decreased neuropathic pain compared with what was seen using a control vector. Next steps could include testing viral vectors that express the PTH2R antagonist in preclinical models of chronic pain.	Unpatented; a small molecule antagonist of PTH2R is available for licensing	Dimitrov, E.L. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 22, 2013; doi:10.1073/pnas.1306342110 <b>Contact:</b> Ted B. Usdin, National Institute of Mental Health, Bethesda, Md. e-mail: <a href="mailto:usdint@mail.nih.gov">usdint@mail.nih.gov</a>
		<b>SciBX 6(34); doi:10.1038/scibx.2013.933</b> Published online Sept. 5, 2013		
Spinal cord injury (SCI)	Matrix metalloproteinase 9 (MMP9)	Mouse studies suggest antagonizing MMP9 in combination with physical therapy could be useful for treating SCI. In a mouse model of SCI, <i>Mmp9</i> knockout plus physical therapy improved gait and led to lower local inflammation and greater white matter survival than no knockout or no physical therapy. Researchers did not disclose next steps, which could include testing MMP9 inhibitors in rodent models of SCI.	Patent and licensing status undisclosed	Hansen, C.N. <i>et al. J. Neurosci.</i> ; published online Aug. 7, 2013; doi:10.1523/JNEUROSCI.1576-13.2013 <b>Contact:</b> D. Michele Basso, The Ohio State University, Columbus, Ohio e-mail: <a href="mailto:michele.basso@osumc.edu">michele.basso@osumc.edu</a>
		<b>SciBX 6(34); doi:10.1038/scibx.2013.934</b> Published online Sept. 5, 2013		
<b>Other</b>				
Hearing loss	Neurotrophic tyrosine kinase receptor 2 (NTRK2; TrkB)	<i>In vitro</i> and mouse studies suggest TrkB agonists could help treat hearing loss. In neurotrophin-depleted cochlear cultures, TrkB agonists increased survival of spinal ganglion neurons (SGNs) compared with no treatment. In patch-clamp studies on auditory SGNs, pretreatment with TrkB agonists increased currents compared with no treatment. In a mouse model of deafness, TrkB agonists decreased electrically evoked auditory response thresholds and increased SGN density. Next steps include preclinical and IND-enabling studies of a TrkB agonist prodrug.	Patent application filed; available for licensing	Yu, Q. <i>et al. J. Neurosci.</i> ; published online Aug. 7, 2013; doi:10.1523/JNEUROSCI.0854-13.2013 <b>Contact:</b> Xi Lin, Emory University School of Medicine, Atlanta, Ga. e-mail: <a href="mailto:xlin2@emory.edu">xlin2@emory.edu</a> <b>Contact:</b> Keqiang Ye, same affiliation as above e-mail: <a href="mailto:kye@emory.edu">kye@emory.edu</a>
		<b>SciBX 6(34); doi:10.1038/scibx.2013.935</b> Published online Sept. 5, 2013		
<b>Transplantation</b>				
Graft-versus-host disease (GvHD)	Protein kinase C $\alpha$ (PRKCA); protein kinase C $\theta$ (PRKCQ)	Mouse studies suggest dual inhibition of PRKCA and PRKCQ could help prevent GvHD. In a mouse model of myeloablative hematopoietic cell transplantation, a dual inhibitor of PRKCA and PRKCQ decreased donor T cell proliferation, migration, chemokine and cytokine production and GvHD compared with vehicle. Next steps include testing the dual inhibitor in xenograft models of GvHD and analyzing its pharmacokinetics in primates.	Patent filed by Rigel Pharmaceuticals Inc.; available for licensing	Haarberg, K.M.K. <i>et al. Blood</i> ; published online Aug. 1 2013; doi:10.1182/blood-2012-12-471938 <b>Contact:</b> Xue-Zhong Yu, H. Lee Moffitt Cancer Center & Research Institute, Tampa, Fla. e-mail: <a href="mailto:yux@muscc.edu">yux@muscc.edu</a>
		<b>SciBX 6(34); doi:10.1038/scibx.2013.936</b> Published online Sept. 5, 2013		

## 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
<b>Chemistry</b>			
Biosynthesis of GE2270 and derivatives in <i>Nonomuraea</i>	Biosynthesis of GE2270 in a strain of <i>Nonomuraea</i> bacteria could lead to the development of improved methods for synthesizing the thiopetide macrocycle and creating derivatives. GE2270 and its derivatives have potent activity against Gram-positive bacteria, but the bacteria that produces it, <i>Planobispora rosea</i> , is not amenable to genetic manipulation. The <i>P. rosea</i> gene cluster responsible for GE2270 synthesis was expressed in a strain of <i>Nonomuraea</i> that is amenable to genetic manipulation. In the transgenic <i>Nonomuraea</i> strain, manipulation of the GE2270 gene cluster resulted in the generation of four fully cyclized GE2270 variants. Next steps could include generating additional GE2270 variants and optimizing their potency and pharmacokinetic properties.	Patent application filed; licensing status unavailable	Tocchetti, A. <i>et al. Chem. Biol.</i> ; published online Aug. 8, 2013; doi:10.1016/j.chembiol.2013.07.005 <b>Contact:</b> Margherita Sosio, Naicons scarl, Milan, Italy e-mail: <a href="mailto:msosio@naicons.com">msosio@naicons.com</a>
	<b>SciBX 6(34); doi:10.1038/scibx.2013.937</b> Published online Sept. 5, 2013		
Fourteen-step synthesis of ingenol to aid analog design	A 14-step process for ingenol synthesis could facilitate the generation of analogs for therapeutic evaluation. Ingenol is a diterpenoid, and analogs of the molecule are marketed to treat cancer. However, ingenol currently is obtained directly from its plant source with an isolation yield of about 0.028% by weight. In the new approach, a 2-phase, 14-step chemical synthesis, starting from the inexpensive commodity chemical 3-carene, generated ingenol with a 1.2% overall yield. Next steps include optimizing the final steps of the process and trying to further decrease the number of synthesis steps. Leo Pharma A/S markets Picato ingenol mebutate gel, an ingenol 3-angeloyl topical formulation, to treat actinic keratosis. The topical drug also is in Phase II testing to treat basal cell carcinoma (BCC).	Provisional patent application filed; licensing details available from Leo Pharma	Jørgensen, L. <i>et al. Science</i> ; published online Aug. 1, 2013; doi:10.1126/science.1241606 <b>Contact:</b> Phil S. Baran, The Scripps Research Institute, La Jolla, Calif. e-mail: <a href="mailto:pbaran@scripps.edu">pbaran@scripps.edu</a> <b>Contact:</b> Steve J. McKerrall, same affiliation as above e-mail: <a href="mailto:stevenmc@scripps.edu">stevenmc@scripps.edu</a>
	<b>SciBX 6(34); doi:10.1038/scibx.2013.938</b> Published online Sept. 5, 2013		
<b>Disease models</b>			
Mouse model of atopic dermatitis-like inflammation	A transgenic mouse model of atopic dermatitis could be useful for studying disease pathogenesis and identifying new treatments for the condition. In the model, mice were engineered to overexpress Il-33 (Nf- $\kappa$ B) in the skin and developed spontaneous itchy dermatitis at 6–8 weeks of age. The mice developed skin lesions that recapitulated the markers of atopic dermatitis in humans, including increased mast cell, histamine and IgE levels, whereas skin from wild-type controls did not. In the mouse model, a histamine receptor antagonist decreased scratching behaviors compared with no treatment. Next steps could include using the model to evaluate therapeutic candidates for atopic dermatitis.	Patent and licensing status unavailable	Imai, Y. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Aug. 5, 2013; doi:10.1073/pnas.1307321110 <b>Contact:</b> Kiyofumi Yamanishi, Hyogo College of Medicine, Nishinomiya, Japan e-mail: <a href="mailto:kyamanis@hyo-med.ac.jp">kyamanis@hyo-med.ac.jp</a> <b>Contact:</b> Kenji Nakanishi, same affiliation as above e-mail: <a href="mailto:nakaken@hyo-med.ac.jp">nakaken@hyo-med.ac.jp</a>
	<b>SciBX 6(34); doi:10.1038/scibx.2013.939</b> Published online Sept. 5, 2013		
Mouse model of sympathetic nervous system activation in metabolic syndrome	A new mouse model could help guide development of therapies that target the sympathetic nervous system in metabolic syndrome. In mice, knockout of the <i>vav 3 oncogene</i> ( <i>Vav3</i> ) guanine nucleotide exchange factor-encoding gene led to liver steatosis and diabetes under a normal diet but protected from obesity and diabetes under a high-fat diet. <i>Vav3</i> knockout mice showed chronic activation of the noradrenergic system, and pharmacological inhibition of adrenergic receptors prevented the onset of metabolic symptoms. Next steps could include using the mouse model to study the interdependence between diet and chronic activation of the sympathetic nervous system.	Patent and licensing status unavailable	Menacho-Márquez, M. <i>et al. Cell Metab.</i> ; published online Aug. 6, 2013; doi:10.1016/j.cmet.2013.07.001 <b>Contact:</b> Xosé R. Bustelo, University of Salamanca, Salamanca, Spain e-mail: <a href="mailto:xbustelo@usal.es">xbustelo@usal.es</a>
	<b>SciBX 6(34); doi:10.1038/scibx.2013.940</b> Published online Sept. 5, 2013		

## This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Transgenic, humanized mouse model of HCV infection	Partially immunocompromised, humanized mice could be used as a model of HCV infection to help evaluate antiviral drug candidates. Transgenic mice with stable expression of human CD81 and occludin (OCLN) in the liver supported HCV entry, but infection was limited to a single cycle. In these transgenic mice, deletion of <i>signal transducer and activator of transcription 1 (Stat1)</i> rendered the animals partially immunocompromised and susceptible to HCV entry, infection and replication. Next steps include selecting viral variants that replicate more robustly in a series of mouse strains with progressively compromised immune systems to establish a fully competent mouse model of HCV infection. Apath LLC is in discussion with The Jackson Laboratory to make the mouse model available to the research community.  <b>SciBX 6(34); doi:10.1038/scibx.2013.941</b> <b>Published online Sept. 5, 2013</b>	Use of OCLN in the mouse model of HCV patented by Apath; available for licensing from Apath <b>Contact:</b> Robert Roth, Apath LLC, St. Louis, Mo. e-mail: <a href="mailto:aproth@apath.com">aproth@apath.com</a>	Dorner, M. <i>et al. Nature</i> ; published online July 31, 2013; doi:10.1038/nature12427 <b>Contact:</b> Alexander Ploss, Princeton University, Princeton, N.J. e-mail: <a href="mailto:aploss@princeton.edu">aploss@princeton.edu</a> <b>Contact:</b> Charles M. Rice, The Rockefeller University, New York, N.Y. e-mail: <a href="mailto:ricec@rockefeller.edu">ricec@rockefeller.edu</a>
<b>Drug platforms</b>			
2'-O-methyltransferase-deficient, live, attenuated dengue virus vaccine	2'-O-methyltransferase-deficient dengue virus could be used to develop live, attenuated vaccines against the infection. In mice and nonhuman primates, dengue virus serotypes 1 and 2 with loss-of-function mutations in 2'-O-methyltransferase were attenuated but still induced a protective immune response, including the production of neutralizing antibodies. In mice and nonhuman primates, vaccination with an attenuated dengue strain protected the animals from a subsequent challenge with a virulent strain. Next steps include evaluating the 2'-O-methyltransferase deficiency in dengue virus serotypes 3 and 4 and then developing a vaccine candidate that could protect against all four dengue virus serotypes.  <b>SciBX 6(34); doi:10.1038/scibx.2013.942</b> <b>Published online Sept. 5, 2013</b>	Patent application filed; available for licensing from the Agency for Science, Technology and Research (A*STAR)	Züst, R. <i>et al. PLoS Pathog.</i> ; published online Aug. 1, 2013; doi:10.1371/journal.ppat.1003521 <b>Contact:</b> Katja Fink, Agency for Science, Technology and Research (A*STAR), Singapore e-mail: <a href="mailto:katja_fink@immunol.a-star.edu.sg">katja_fink@immunol.a-star.edu.sg</a> <b>Contact:</b> Pei-Yong Shi, Novartis Institute for Tropical Diseases, Singapore e-mail: <a href="mailto:pei_yong.shi@novartis.com">pei_yong.shi@novartis.com</a> <b>Contact:</b> Cheng-Feng Qin, Beijing Institute of Microbiology & Epidemiology, Beijing, China e-mail: <a href="mailto:qincf@bmi.ac.cn">qincf@bmi.ac.cn</a>
Small molecules that prevent teratoma formation in human pluripotent stem cell-derived therapies	Small molecules that are selectively toxic to human pluripotent stem cells could help decrease the tumorigenicity risk of stem cell-derived therapies. Residual, undifferentiated stem cells in stem cell-derived cell therapies can lead to teratoma formation. In mixed cultures of differentiated human cells and undifferentiated human stem cells, small molecule inhibitors of survivin (BIRC5), such as sepantronium, and small molecule inhibitors of B cell CLL lymphoma 10 (BCL10) selectively induced apoptosis in undifferentiated cells. In a mixture of differentiated human cells and undifferentiated human stem cells injected into mice, none of the cell mixtures pretreated with sepantronium or another small molecule survivin inhibitor developed teratomas, whereas all the untreated cell mixtures did. Next steps include developing more specific inhibitors of survivin and BCL10. Astellas Pharma Inc.'s sepantronium, a survivin expression inhibitor, is in Phase II testing to treat non-Hodgkin's lymphoma (NHL). Erimos Pharmaceutical LLC's Terameprocol tetra-O-methyl nordihydroguaiaretic acid, a small molecule inhibitor of the production and activation of survivin, is in Phase I for multiple cancers.  <b>SciBX 6(34); doi:10.1038/scibx.2013.943</b> <b>Published online Sept. 5, 2013</b>	Patent application filed; unavailable for licensing	Lee, M.-O. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Aug. 5, 2013; doi:10.1073/pnas.1303669110 <b>Contact:</b> Hyuk-Jin Cha, Sogang University, Seoul, South Korea e-mail: <a href="mailto:hjcha@sogang.ac.kr">hjcha@sogang.ac.kr</a> <b>Contact:</b> Kwang-Soo Kim, Harvard Medical School, Boston, Mass. e-mail: <a href="mailto:kskim@mclean.harvard.edu">kskim@mclean.harvard.edu</a>

## This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Transcription activator-like effector nucleases (TALENs) to eliminate mitochondria-specific mutant DNA	TALENs could be useful for treating mitochondrial disease by selectively removing mutant mitochondrial DNA (mtDNA). In cells with a pathogenic point-of-deletion mutation in mtDNA, transient transfection of plasmids expressing mitochondria-targeted TALENs decreased levels of the mutant but not wild-type mtDNA. Next steps include developing efficient TALEN delivery strategies and testing the method in mouse models of mitochondrial disease.  <b>SciBX 6(34); doi:10.1038/scibx.2013.944</b> Published online Sept. 5, 2013	Patenting status undisclosed; licensing details available from the University of Miami	Bacman, S.R. <i>et al. Nat. Med.</i> ; published online Aug. 4, 2013; doi:10.1038/nm.3261 <b>Contact:</b> Carlos T. Moraes, University of Miami, Miami, Fla. e-mail: <a href="mailto:cmoraes@med.miami.edu">cmoraes@med.miami.edu</a>
<b>Imaging</b>			
Noninvasive, 3D imaging of retinal and choroidal vasculature using phase-variance optical coherence tomography	A noninvasive method to image vasculature in the eye could help monitor treatment responses and diagnose patients who have age-related macular degeneration (AMD) and other choroidal vascular diseases. The method couples noninvasive, high-resolution optical coherence tomography with phase variance-based analysis to generate 3D images of vasculature and circulation in the retina and three outermost layers of the choroid of the human eye. In the eye of a patient with AMD who had geographic atrophy, the imaging method identified vascular abnormalities in the retina and choroid and distinguished between healthy and atrophic regions of the macula. Next steps could include using the method to monitor treatment responses in patients who have AMD.  <b>SciBX 6(34); doi:10.1038/scibx.2013.945</b> Published online Sept. 5, 2013	Patent and licensing status unavailable	Kim, D.Y. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Aug. 5, 2013; doi:10.1073/pnas.1307315110 <b>Contact:</b> John S. Werner, University of California, Davis, Calif. e-mail: <a href="mailto:jswerner@ucdavis.edu">jswerner@ucdavis.edu</a> <b>Contact:</b> Scott E. Fraser, California Institute of Technology, Pasadena, Calif. e-mail: <a href="mailto:sefraser@caltech.edu">sefraser@caltech.edu</a>
<b>Markers</b>			
Ribosomal protein S6 kinase (RSK) phosphorylation to predict response to BRAF, MEK or phosphoinositide 3-kinase (PI3K) inhibitors	Studies in patient samples, cell culture and mice suggest RSK phosphorylation status could predict response to BRAF, MEK or PI3K inhibitors. In a panel of cancer cell lines, reduction of RSK phosphorylation after treatment with a MEK or BRAF inhibitor was a stronger predictor of sensitivity to treatment than previously reported predictive markers, including <i>PTEN</i> ( <i>MMAC1</i> ; <i>TEP1</i> ) loss or protein kinase B (PKB; PKBA; AKT; AKT1) phosphorylation. In a patient treated with both a MEK and BRAF inhibitor, RSK phosphorylation was eliminated prior to a complete response, and subsequent tumor recurrence was associated with restoration of RSK phosphorylation. In mouse xenograft models, tumors resistant to a phosphatidylinositol 3-kinase catalytic subunit $\alpha$ -polypeptide (PIK3CA; p110 $\alpha$ ) inhibitor had higher levels of RSK phosphorylation than sensitive tumors. In these models, adding an mammalian target of rapamycin (mTOR; FRAP; RAFT1) inhibitor decreased RSK phosphorylation and tumor growth compared with adding vehicle. Ongoing work includes clinical trials combining mTOR inhibitors with PI3K, RAF or MEK inhibitors.  <b>SciBX 6(34); doi:10.1038/scibx.2013.946</b> Published online Sept. 5, 2013	Patent and licensing status unavailable	Corcoran, R.B. <i>et al. Sci. Transl. Med.</i> ; published online July 31, 2013; doi:10.1126/scitranslmed.3005753 <b>Contact:</b> Jeffrey A. Engelman, Massachusetts General Hospital Cancer Center, Boston, Mass. e-mail: <a href="mailto:jengelman@partners.org">jengelman@partners.org</a> <b>Contact:</b> Daniel A. Haber, same affiliation as above e-mail: <a href="mailto:dhaber@partners.org">dhaber@partners.org</a>  Elkabets, M. <i>et al. Sci. Transl. Med.</i> ; published online July 31, 2013; doi:10.1126/scitranslmed.3005747 <b>Contact:</b> José Baselga, Memorial Sloan-Kettering Cancer Center, New York, N.Y. e-mail: <a href="mailto:baselgaj@mskcc.org">baselgaj@mskcc.org</a> <b>Contact:</b> Maurizio Scaltriti, same affiliation as above e-mail: <a href="mailto:scaltrim@mskcc.org">scaltrim@mskcc.org</a>

**Corrigendum: Analysis: Cover Story**Baas, T. *SciBX* 6(33); doi:10.1038/scibx.2013.879

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In the Analysis item “Sleuthing for toxicity,” several quotes from Michel Sadelain have been updated in the interest of accuracy at the request of the researcher.

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