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THIS WEEK

ANALYSIS

COVER STORY

TARGETS & MECHANISMS

TOOLS

THE DISTILLERY

INDEXES

Connecting the microbiome to obesity-associated cancers

By Kai-Jye Lou, Senior Writer

Multiple epidemiological studies have shown associations between obesity and increased risk for various cancers, [1,2] but the mechanisms underlying the interplay of the two conditions have been poorly understood. New research from Japan suggests obesity-induced changes in the gut microbiome could be one potential culprit,[3] providing new directions to develop microbiome-targeted diagnostics and interventions.

A team led by Eiji Hara, chief of the Division of Cancer Biology at the **Japanese Foundation for Cancer Research**, has traced the association between obesity and increased cancer risk to gut microbiota communities that produce a DNA-damaging bile acid. The work also elucidates the role of cellular senescence in cancer, something Hara has been studying for the past decade.

Senescence typically is viewed as a tumor-suppressive mechanism, but recent studies by Hara and others have found that senescent cells can take on a secretory phenotype and produce and release inflammatory factors as well as growth factors.[4–6]

Some of the factors secreted by these senescent cells have been associated with increased cancer risk in obesity. Hara's group sought to determine whether cells that take on the senescence-associated secretory phenotype could be an underlying contributor to the increased cancer risk in obesity.

To map out a mechanistic pathway linking obesity to cancer, the researchers carried out a series of studies in mice exposed to a carcinogen that rendered them prone to developing hepatocellular carcinoma (HCC) when obese but not when lean. The obese mice showed increased numbers of hepatic stellate cells with the senescence-associated secretory phenotype and had higher levels of deoxycholic acid, a bile acid produced by certain microbial strains in the gut.

Based on their findings, the researchers hypothesized that obesity can increase the production of deoxycholic acid in select populations of gut bacteria, which in turn increases the appearance of senescent hepatic stellate cells that secrete inflammatory factors and drive the development of HCC (*see* **Figure 1, "Model for obesity-associated liver cancer driven by the gut microbiome"**).

Results were published in *Nature*.

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Alterations in gut microbiota have been associated with cancer,[7] inflammation[8] and obesity.[9] The bile acid deoxycholic acid is a metabolite produced by some strains of bacteria in the gut. The acid causes DNA damage[10] and enhances liver carcinogenesis.[11]

Last year, researchers at **Columbia University** published data suggesting gut microbiota and signaling through toll-like receptors can drive inflammatory and fibrogenic responses that contribute to carcinogenesis in the liver.[12]

> **"The importance of the current study is that it gives us an understanding of how these three seemingly disparate phenomena are mechanistically linked with one another."**
> ***—Judith Campisi, Buck Institute***

"The importance of the current study is that it gives us an understanding of how these three seemingly disparate phenomena are mechanistically linked with one another," said Judith Campisi, a professor at the **Buck Institute** and a senior scientist at the **Lawrence Berkeley National Laboratory**. "My suspicion, though, is that this will be one of multiple mechanisms that can drive obesity-associated cancers."

Campisi noted that there could be other bile acids that drive obesity-associated cancers as well.

"The current study helps set the stage for identifying the types of bugs and metabolites that could be important to obesity-associated HCC," said Peter DiStefano, SVP of R&D at **Second Genome Inc.** "However, the researchers will need to go beyond enumeration and on to characterizing the functional role of these bugs and metabolites in the context of the disease."

Second Genome is developing therapies that can alter the composition and activity of microbial communities in the body. In June, the company partnered with **Johnson & Johnson**'s Janssen Biotech Inc. unit to characterize the role of bacterial populations in the human gut in ulcerative colitis (UC) and identify potential drug targets.

Exploring opportunities

Hara said his group is planning studies to determine whether the results in the mouse model will translate into humans.

Such studies, he said, would include collecting clinical samples and using them to determine whether levels of deoxycholic acid or deoxycholic acid–producing bacteria are higher in obese individuals than in nonobese individuals.

"If our mouse findings translate into humans, one can imagine the possibility of developing methods to predict obesity-associated cancer risk in the general population, for example, by measuring the levels of deoxycholic acid or deoxycholic acid–producing bacteria in fecal samples," Hara told *SciBX*. He expects the group still is three to four years away from developing such a method.

Campisi said the findings provide general ideas on potential therapeutic and interventional strategies but expects the near-term application of Hara's work is likely to be in the diagnostics space.

"The first area of opportunity is that we can now think about ways to identify and treat obese people who are at risk of cancer based on targeting the gut microbiota," she told *SciBX*. "A second area one may want to look for therapeutic opportunities is in identifying and

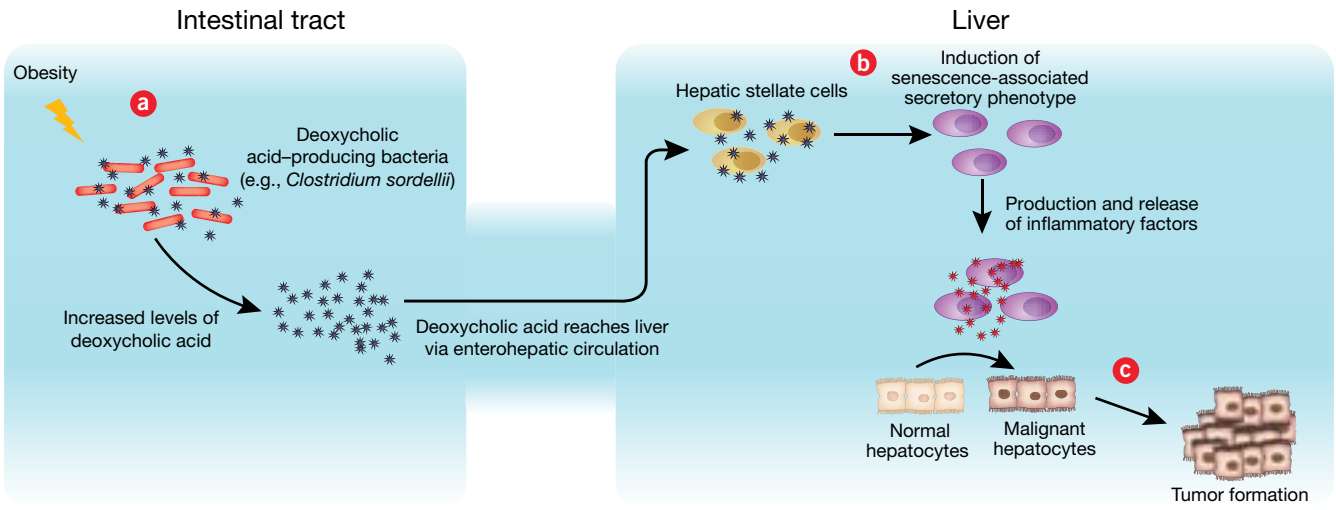

Figure 1. Model for obesity-associated liver cancer driven by the gut microbiome. Obesity is associated with increased risk for various cancers, but the mechanisms underlying the greater risk are unclear. As reported in Yoshimoto *et al.*, obesity appears to drive the development of hepatocellular carcinoma (HCC) through the microbiome.

[**a**] According to the proposed model, obesity results in changes in the gut microbiota that lead to increased levels of deoxycholic acid. [**b**] Molecules of this DNA-damaging bile acid travel via enterohepatic circulation to the liver and induce the senescence-associated secretory phenotype in hepatic stellate cells. [**c**] Inflammatory factors released by the senescent hepatic stellate cells promote malignant transformation in nearby hepatocytes, which leads to tumor formation and HCC.

interfering with enzymes that mediate the production of deoxycholic acid or other bile acids that could increase cancer risk. A third area of opportunity is in identifying and evaluating compounds that specifically target senescent cells."

"One can imagine the possibility of developing methods to predict obesity-associated cancer risk in the general population, for example, by measuring the levels of deoxycholic acid or deoxycholic acid–producing bacteria in fecal samples."

—Eiji Hara, Japanese Foundation for Cancer Research

Campisi wanted to see studies that identify additional gut microbiome–derived metabolites that are able to induce cellular senescence and what distal tissues they might affect.

DiStefano agreed that it is still too early to pursue specific therapeutic strategies based on the reported findings, but he did note that Hara's work supports the notion that potential targets for diseases in host tissues could reside in microbes.

In addition to determining whether the described mechanism in mice will translate into human systems, DiStefano said it will be important to identify bacterial strains that can alter the amount of deoxycholic acid and to investigate how altering levels of the metabolite will affect the phenotype in HCC and other disease settings.

The Japanese Foundation for Cancer Research has filed a patent covering the reported findings. The work is available for licensing and collaboration. Hara said his group is specifically interested in collaborating with others to develop methods to prevent the growth of bacteria that produce deoxycholic acid and strategies to identify high-risk individuals based on measuring deoxycholic acid levels.

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REFERENCES

1. Calle, E.E. & Kaaks, R. *Nat. Rev. Cancer* **4**, 579–591 (2004)
2. Khandekar, M.J. *et al. Nat. Rev. Cancer* **11**, 886–895 (2011)
3. Yoshimoto, S. *et al. Nature*; published online June 26, 2013; doi:10.1038/nature12347
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4. Kuilman, T. & Peeper, D.S. *Nat. Rev. Cancer* **9**, 81–94 (2009)
5. Rodier, F. & Campisi, J. *J. Cell Biol.* **192**, 547–556 (2011)
6. Takahashi, A. *et al. Mol. Cell* **45**, 123–131 (2012)
7. Arthur, J.C. *et al. Science* **338**, 120–123 (2012)
8. Kamada, N. *et al. Nat. Rev. Immunol.* **13**, 321–335 (2013)
9. Ley, R.E. *et al. Nature* **444**, 1022–1023 (2006)
10. Payne, C.M. *et al. Carcinogenesis* **28**, 215–222 (2007)
11. Kitazawa, S. *et al. Carcinogenesis* **11**, 1323–1328 (1990)
12. Dapito, D.H. *et al. Cancer Cell* **21**, 504–516 (2012)

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Prostate cancer's nerves

By Lauren Martz, Staff Writer

In prostate cancer, a handful of companies are racing to develop third-generation antiandrogen therapeutics that overcome resistance mechanisms that render first- and second-generation molecules ineffective.[1,2] The problem is that any direct attack on the tumor is likely to lead to resistance mutations in a short stretch of time.

Now, a team from the **Albert Einstein College of Medicine of Yeshiva University** has taken a new approach for treating the disease. The group has evidence that blocking nerve signaling pathways with marketed β-adrenergic receptor and muscarinic receptor antagonists can inhibit prostate tumor initiation and metastasis.[3] The team plans to study the drugs in the new indication, although it is not yet clear whether the approach can treat established disease.

Previous studies have reported a link between prostate cancer progression and nervous system stimulation. For example, a 2001 study found that sensory neurons stimulate prostate cancer proliferation *in vitro*.[4]

This year, researchers at **Oslo University Hospital** reported that patients with prostate cancer who were taking β-blockers had a lower incidence of recurrence and mortality than patients who did not take the medication.[5,6]

The Albert Einstein College of Medicine team inhibited sympathetic nerve signaling in mice by knocking out *adrenergic receptor* β_2 (*Adrb2*) and *Adrb3* and saw less tumor initiation than that in wild-type controls.

In addition, inhibition of parasympathetic nerve signaling by blocking muscarinic acetylcholine receptor M1 (CHRM1; HM1) helped block tumor invasion and metastasis.

β-Blockers are marketed to treat cardiovascular conditions including hypertension, myocardial infarction (MI) and heart failure. CHRM1 antagonists are marketed to treat incontinence and drooling.

Building on the previous work, Claire Magnon, Paul Frenette and colleagues set out to determine whether blocking signaling of the autonomic nervous system in the prostate could help treat or prevent the disease.

Magnon is an assistant professor at the Albert Einstein College of Medicine. Frenette is director and chair of the Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research and a professor of medicine and cell biology at the college. The group also included researchers from **Duke University**, the **Icahn School of Medicine at Mount Sinai** and the **Durham VA Medical Center**.

The team injected luciferase-expressing human prostate cancer cells into the prostates of immunocompromised mice to bioluminescently model the disease. The number of tumor-infiltrating sympathetic plus parasympathetic nerve fibers within the tumor increased with cancer development, which suggested the tumors recruited new nerves to the stroma.

In the mice, elimination of sympathetic nerve fibers prevented development of prostate cancers.

The group then hypothesized that because sympathetic neurons signal through β-adrenergic receptors, antagonizing the receptors might help prevent prostate cancer. Indeed, genetic deletion of *Adrb2* or *Adrb3* in the mice delayed tumor development. The effect was even more pronounced in double knockout animals.

Parasympathetic signaling also played a role in tumor development and progression.

In the bioluminescent mouse model, a nonspecific agonist of muscarinic cholinergic receptors, which are activated by parasympathetic neurons, increased tumor cell invasion into lymph nodes compared with no treatment. A nonspecific CHRM antagonist, a specific CHRM1 antagonist or genetic *Chrm1* knockout prevented the increase in invasion, decreased metastasis and improved survival.

Finally, an analysis of prostate tumor and tissue samples from 43 treatment-naïve patients showed that high nerve fiber densities in and around tumors correlated with poor prognosis.

Results were published in *Science*.

> **"Drug resistance is a major problem in cancer drug development. Assuming that the high plasticity of the cancer cells makes them more prone to develop resistance, targeting the stromal cells like the nerves instead may slow down or block the resistant development."**
>
> ***—Kristin Austlid Taskén, Oslo University Hospital***

Drug repurposing

Researchers agree that β-adrenergic receptor antagonists and CHRM1 antagonists could be repurposed to prevent and possibly treat prostate cancer.

Ian Barron, a postdoctoral research fellow at **Trinity College Dublin**, told *SciBX* that "a number of drugs that inhibit these targets are already available. Their safety profiles are well understood."

"Since such blockers are in routine clinical use, their employment in prostate cancer treatment should be relatively seamless," added Norman Maitland, director of the YCR Cancer Research Unit and a professor of molecular biology at **The University of York**.

Maitland and Kristin Austlid Taskén, an adjunct professor of urology at Oslo University Hospital, both liked that the antagonists target the nerves in the tumor microenvironment rather than the tumor cells themselves. Taskén was the group leader on the retrospective study linking β-blockers with improved prostate cancer prognosis.

Androgen receptor–targeting drugs and chemotherapies for prostate cancer target the cancer cells themselves. "Drug resistance is a major problem in cancer drug development. Assuming that the high plasticity of the cancer cells makes them more prone to develop resistance, targeting the stromal cells like the nerves instead may slow down or block the resistant development," said Taskén.

An unanswered question is whether blocking nerve signaling pathways can treat established disease.

"It is difficult to see how this mechanism of action fits into a postdiagnosis treatment paradigm, as by the time that most patients present with their cancer, tumor dissemination will have already happened," said Barron.

(Continues on page 5)

Activating memory

By Lev Osherovich, Senior Writer

Despite more than a decade of research suggesting histone acetyltransferases play a role in learning and memory, the targets have proven difficult to selectively activate in the brain. Now, a French and Indian team has preclinical proof of concept for activating histone acetyltransferases with a small molecule formulated to cross the blood brain barrier.[1] The compound enhanced brain activity in wild-type mice, but its selectivity and utility in neurodegenerative disease remain to be seen.

Histone acetyltransferases (HATs) are a family of epigenetic regulators that, in the brain, broadly modulate gene expression in response to neural stimulation. HATs work by adding acetyl groups to histones, which are nuclear proteins that package and organize chromosomal DNA. Acetylation generally causes tightly wound and transcriptionally silent chromatin to loosen up and become active.

The new report describes the molecular and behavioral effects of activating two HATs linked to learning and memory—CREB binding protein (CREBBP; CBP) and a related protein called E1A binding protein p300 (EP300; p300).

In the late 1990s, academic researchers identified CBP as a critical regulator of learning and memory in sea slugs, flies and mice.[2,3] Despite genetic evidence that activating CBP and other HATs can improve brain function, identifying small molecule activators of enzymes is a tall order and for HATs is compounded by the blood brain barrier's exclusion of most chemical classes from the CNS.

Researchers at the **Jawaharlal Nehru Centre for Advanced Scientific Research** and the **University of Strasbourg** have made progress on both the chemistry and delivery fronts and have designed a HAT-activating compound that appears to work in mice.

"Our results, obtained *in vivo*, show that new HAT activator molecules provide therapeutic options for brain diseases," said Anne-Laurence Boutillier, the team's coleader and the director of research at **Centre National de la Recherche Scientifique** (CNRS) at the University of Strasbourg.

The team began by making brain-permeating derivatives of an EP300-activating compound previously identified in the laboratory of the team's coleader Tapas Kundu.

Kundu is a professor in the Transcription and Disease Laboratory at the Jawaharlal Nehru Centre.[4]

The researchers first synthesized a molecule, TTK21, that increased EP300 and CBP activity *in vitro* compared with vehicle control. To increase TTK21's ability to enter cells, the team conjugated the molecule to carbon nanospheres.

Cultured neuroblastoma cells treated with TTK21-covered

(Continues on page 6)

(Continued from "**Prostate cancer's nerves**," p. 4)

Maitland added that most prostate cancer chemotherapies are used when the disease has penetrated the prostatic capsule or the cancer cells have metastasized to the local lymph nodes.

"Would such a nervous system treatment affect these cells at all? By this time, the tumors have become largely independent of the prostate stroma influence and have adapted to the bone environment, for example," he said.

Despite the concerns, Magnon expects that ADRB2, ADRB3 and CHRM1 inhibitors may prevent and treat both early and late stage cancers.

"Targeting the sympathetic nervous system may help to treat early prostate cancer. However, after surgery of advanced cancers, administration of selective β-blockers targeting ADRB2 and ADRB3 might block potential recurrence at the primary site. Adjunction of a CHRM1 inhibitor may prevent perioperative tumor dissemination and then metastasis," she said.

Hagop Youssoufian, EVP of R&D at **Progenics Pharmaceuticals Inc.**, thinks the best uses for nervous system–targeting therapeutics will likely be in patients predisposed to prostate cancer, patients who had primary tumors removed by surgery and patients with minimal residual disease after primary therapy.

Progenics' PSMA ADC, an antibody-drug conjugate targeting prostate-specific membrane antigen (PSMA; FOLH1; GCPII), is in Phase II testing to treat prostate cancer.

Magnon thinks combining ADRB2, ADRB3 and CHRM1 antagonists plus chemotherapy could treat established disease while preventing recurrence. Before studying such a combination in mice, Magnon hopes to better characterize the nerve signaling processes in the tumor microenvironment.

"I would like to dissect further nerve interactions with other components of the stroma in order to design the best targeted therapies," she said.

Magnon said the Albert Einstein College of Medicine has patented the work and the IP is available for licensing.

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REFERENCES

1. Joseph, J.D. *et al. Cancer Discov.*; published online June 18, 2013; doi:10.1158/2159-8290.CD-13-0226
2. Donner, A. *SciBX* **6**(25); doi:10.1038/scibx.2013.615
3. Magnon, C. *et al. Science*; published online July 12, 2013; doi:10.1126/science.1236361
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4. Ayala, G.E. *et al. Prostate* **49**, 213–233 (2001)
5. Grytli, H.H. *et al. Prostate* **73**, 250–260 (2013)
6. Grytli, H.H. *et al. Eur. Urol.*; published online Jan. 14, 2013; doi:10.1016/j.eururo.2013.01.007

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Oslo University Hospital, Oslo, Norway
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Trinity College Dublin, Dublin, Ireland
The University of York, York, U.K.

nanoparticles had higher levels of histone acetylation than controls receiving unconjugated nanoparticles.

In mice, intraperitoneal injection of TTK21 particles led to their accumulation in the brain, liver and spleen. The nanoparticles were eliminated from the liver and spleen in a matter of days but substantial amounts remained in the brain for up to two weeks. Effects on spleen and liver function were not reported.

Animals receiving the molecule had higher levels of histone acetylation in the cortex and hippocampus than nanoparticle carrier–treated controls, resulting in greater expression of genes associated with neuronal growth. The functional consequence of TTK21 treatment was increased neurogenesis and better performance in assays of memory duration compared with controls receiving drug-free nanoparticles.

Results were reported in *The Journal of Neuroscience*. TTK21 is the subject of a pending patent in India and is available for licensing.

"Targeting the sympathetic nervous system may help to treat early prostate cancer. However, after surgery of advanced cancers, administration of selective β-blockers targeting ADRB2 and ADRB3 might block potential recurrence at the primary site. Adjunction of a CHRM1 inhibitor may prevent perioperative tumor dissemination and then metastasis."

—Claire Magnon, Albert Einstein College of Medicine of Yeshiva University

HAT trick

The findings suggest TTK21 could be a starting point for the development of a brain-permeating chemical class that activates HATs. However, substantial questions remain about the compound's selectivity and applicability in disease.

"Molecules that favor and promote the *in vivo* maturation and differentiation of newly generated neurons in the adult present an obvious advantage in several neurodegenerative diseases," said Boutillier, citing Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) as potential indications in which to try TTK21.

Her team is focusing on AD. Prior work by other researchers has shown that overexpression of CBP enhances cognitive performance in a mouse model for AD,[5] so activating the protein with a small molecule could yield similar results.

"We are currently testing this compound in two transgenic AD mouse models, one presenting with microtubule-associated protein-τ (MAPT; TAU; FTDP-17) aggregates and the other one with amyloid plaques," said Boutillier.

"The novel hook is not that activating histone acetyltransferases is good for memory, but rather that they came up with chemistry" that leads to HAT activation, said **Atlas Venture** partner Bruce Booth.

In June, Atlas launched **Rodin Therapeutics Inc.** to pursue epigenetic targets for neurodegenerative disease, with Booth taking on the CEO role. **Johnson & Johnson**'s venture arm, **Johnson & Johnson Development Corp.**, invested an undisclosed sum in Rodin's tranched series A round.

Booth said that TTK21 likely needs further medicinal chemistry optimization of its potency and selectivity. He noted that HATs regulate the acetylation of a multitude of proteins besides histones, so broad-spectrum activation of the class might have undesirable effects.

Although TTK21 could activate CBP and EP300 in an *in vitro* assay in the absence of other enzymes, it is not clear whether these relatively low-affinity compounds can act on other HATs inside cells.

Activating both CBP and EP300 may mean "acetylating hundreds of proteins, so you might have broad effects on tons of genes and tons of proteins," said Booth. "The big question is whether there are other nonhistone targets that are affected. The challenge is to get to the specific compounds with a high selectivity."

One way to test TTK21's selectivity would be to assess the compound's effects in cells or mice lacking Cbp or Ep300.

An alternative to activating HATs is to inhibit histone deacetylases (HDACs), a family of epigenetic regulators that removes acetyl groups from histones. Indeed, researchers at **The University of Texas Southwestern Medical Center** reported in April that inhibiting HDAC2 improves learning and memory in similar assays to those used by Boutillier and Kundu's team.[6]

Booth said Rodin is focusing on CNS diseases including AD but did not disclose the company's specific targets.

"We're not talking about which specific epigenetic targets are in our scope and which ones aren't, but the general area of histone and epigenetic modification, transferase activity, demethylation and acetylation is very much at the heart of what we're doing," said Booth.

Rodin is partnered with **Proteros biostructures GmbH** and with Johnson & Johnson's Janssen Research & Development LLC unit and Boston Innovation Center to develop compounds and assays.

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REFERENCES

1. Chatterjee, S. *et al. J. Neurosci.*; published online June 26, 2013; doi:10.1523/JNEUROSCI.5772-12.2013
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2. Kandel, E.R. *Mol. Brain* **5**, 14 (2012)
3. Gräff, J. *et al. Physiol. Rev.* **91**, 603–649 (2011)
4. Balasubramanyam, K. *et al. J. Biol. Chem.* **278**, 19134–19140 (2003)
5. Caccamo, A. *et al. Proc. Natl. Acad. Sci. USA* **107**, 22687–22692 (2010)
6. Morris, M.J. *et al. J. Neurosci.* **33**, 6401–6411 (2013)

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The liver's new bud

By Benjamin Boettner, Assistant Editor

Yokohama City University and **Sekisui Medical Co. Ltd.** researchers have produced vascularized human liver tissue that displays promising metabolic and regenerative capacity in mice.[1] The team now aims to scale up the procedure to generate bigger tissue segments and will test the treatment for liver failure in larger animals.

The human liver has the distinct capacity to regenerate itself. However, acute liver failure or chronic liver damage, which can stem from alcohol abuse, HBV or HCV, can reduce the amount of functional liver tissue to less than 10%–15% of the original functional liver mass.

Below that threshold, intrinsic regeneration does not occur.[2] This point of no return, paired with the relative scarcity of transplantable livers, has spurred research into generating liver tissue *de novo* that does not rely on functional tissue remaining in patients.

Up to now, efforts to regenerate liver tissue have focused on two main strategies. One is the infusion of either autologous bone marrow–derived progenitor cells or G-CSF (CSF3). The former differentiate into mature hepatocytes, whereas the latter promotes the recruitment of bone marrow or blood-derived progenitor cells to the liver.

Phase I trials of both methods have shown improvements in patients with liver cirrhosis.[3]

The other strategy is implantation of more complex, small liver segments generated on artificial or decellularized support structures. Studies by multiple academic groups have shown that recellularized grafts can be implanted into rodents.[3,4]

For both strategies, the drawbacks are short survival times of transplanted cells and structures because of their inability to establish long-lived vascular connectivity and failure to integrate with the host liver.

Now, Takanori Takebe and Hideki Taniguchi, both professors at Yokohama City University, have taken a fresh approach to the problem and created fully vascularized and metabolically active human liver buds *in vitro*.

"In contrast to previous attempts, our approach basically reconstitutes the multiple cellular interactions that are important for liver organogenesis, enabling the cells to self-organize into a three-dimensional tissue presumably emulating a natural spatiotemporal orientation."

—Takanori Takebe, Yokohama City University

The authors turned to induced pluripotent stem (iPS) cells and differentiated them into fetal hepatocytes.[5,6] Coculturing iPS cells with human endothelial cells and mesenchymal stem cells (MSCs) gave rise to self-organizing structures that resembled the first liver buds in the developing human and mouse embryo.

Comparative gene expression analysis revealed largely overlapping patterns between the artificially generated buds and the embryo tissues, underscoring their developmental similarity.

After transplantation into mice, the microvasculature of the liver buds connected to the host vascular system, which enabled the structures to mature into adult, long-lived hepatic tissue capable of performing metabolic reactions. Implantation of 12 liver buds into the mouse mesentery, a well-perfused tissue in the abdominal cavity, extended the survival of animals with failing livers.

"In contrast to previous attempts, our approach basically reconstitutes the multiple cellular interactions that are important for liver organogenesis, enabling the cells to self-organize into a three-dimensional tissue presumably emulating a natural spatiotemporal orientation," said Takebe. "Upon transplantation, cells undergo efficient differentiation into target cell types, experiencing organogenetic inputs from supporting stromal cells, mechanical load and growth stimuli that spatiotemporally correspond to the original developmental stages in the embryo."

The findings were published in *Nature*.

The team carried out the research in collaboration with ADME & Toxicology Research Institute, which is a unit of Sekisui that is focused on custom synthesis and pharmacological testing of compounds.

Long live the liver

Takebe said the team now plans on running studies in nonhuman primates and hopes to enter the clinic in 7–10 years.

Stephen Badylak, deputy director of the **McGowan Institute for Regenerative Medicine** at the **University of Pittsburgh** and a professor in the Department of Surgery at the **University of Pittsburgh Medical Center**, said the study provides proof of concept that complex liver tissue can be grown from scratch.

Although the liver buds are still too small to perfuse a larger tissue segment with blood and at this point lack bile ducts, he said the findings lay the groundwork for a potential new approach to liver transplants.

Badylak, who also is director of the McGowan Institute's Center for Preclinical Tissue Engineering, is generating liver tissue *ex vivo* using decellularized scaffolds as a growth platform for differentiated adult hepatocytes.[7]

"In moving toward the clinic, larger animal models will be essential. It will be important to demonstrate the growth potential, vascular development, bile duct formation and required duration for the iPS cell–derived liver buds to sufficiently enable large liver structures to develop *in vivo*," said Jeffrey Ross, VP of product development at **Miromatrix Inc.**

Miromatrix has licensed worldwide, exclusive rights to numerous patent applications and issued patents from the **University of Minnesota** covering methods of perfusion, decellularizing and recellularizing organs and tissues.

Shay Soker, a professor of regenerative medicine at the **Wake Forest Institute for Regenerative Medicine**, said Takebe's team already has addressed two major challenges: identifying a cell source for liver tissue engineering and having it vascularized *in vivo*. According to Soker, the main remaining hurdle is expanding the size of the liver organoids by 100–1,000-fold while still adequately perfusing the growing tissue mass.

Soker, Ross and Stuart Forbes, a professor of transplantation and regenerative medicine at **The University of Edinburgh**, all think a strategy for achieving balanced growth of the liver bud cell types could be combining the Japanese team's approach with scaffold technology. According to Soker, decellularized matrices generated from adult liver tissue could be seeded with liver bud germinal centers that may expand

and coalesce into a larger, stable structure with improved perfusion and longevity.

In addition to vascularity, larger liver buds will require balanced growth of all three cellular components—differentiating liver cells, endothelial cells and MSCs. On top of that, the organoid's overall structure and functionality will have to be maintained for a long time after implantation.

Forbes thinks the MSC component of the liver buds could be unpredictable. "Ensuring that mesenchymal cells behave how you want them to is a challenge. Would they grow disproportionately faster than hepatocytes when cultured over longer periods of time?" he asked.

He also noted that cells in a chronically damaged liver are permanently exposed to inflammatory signals that may promote unwanted overgrowth of stromal components of liver buds. "We do not know how stable iPS cell–derived cells will be *in vivo* long term, particularly in the setting of ongoing liver injury," said Forbes.

Thus, Forbes thinks acute liver failure might be a better fit for the technology than chronic liver disease. In that setting, he said, "the patient's own liver may regenerate if provided with support during a critical period."

Bud screening

In addition to providing a starting point for developing a next-generation approach to liver transplants, functional liver buds could provide a platform for drug and toxicity screening.

Indeed, Takebe and Taniguchi administered ketoprofen and debrisoquine to mice with transplanted liver buds and observed metabolic reactions specific to human liver cells. The two drugs are metabolized differently by human and mouse hepatocytes and thus allowed the researchers to distinguish the activity of transplanted human hepatocytes from that of the mouse liver's hepatocytes.

"In moving toward the clinic, larger animal models will be essential. It will be important to demonstrate the growth potential, vascular development, bile duct formation and required duration for the iPS cell–derived liver buds to sufficiently enable large liver structures to develop *in vivo*."
—Jeffrey Ross, Miromatrix Inc.

According to Salman Khetani, cofounder of **Hepregen Corp.**, a company developing complex liver cell platforms suitable for high throughput metabolic testing, comprehensive mouse work with a much larger panel of drugs being metabolized by different enzyme systems will be required to find out whether human liver bud activity can be sufficiently differentiated from the mouse liver's metabolism *in vivo*.

Thus, he thinks a more immediate application of liver buds might be their use for *in vitro* drug and toxicity testing.

Badylak also said, "iPS cell–derived liver buds could be generated from not only one human genetic background but also a number of different individual sources. When applied to drug and toxicity testing, such an approach could greatly help to gauge person-to-person variability."

Finally, the approach suggests that regenerative approaches to other organs could be spurred on by iPS cell–derived organ bud technology. As Miromatrix's Ross put it, "Recreating the organ bud–like structure certainly has potential for other organ systems, as it elegantly capitalizes on the very early developmental stage when the morphogenesis of the organ is first put into motion. This could prove an important step in accessing key cell types for future therapies."

Soker specified that mostly modular organs like the liver, pancreas and kidney could benefit from the approach because of their sectional packaging and connectivity to the circulation.

Takebe told *SciBX* that the team has started to evaluate the applicability of the method to the pancreas and kidney.

Takebe and Taniguchi have filed for a patent covering the methodology for generating organ buds for different organs. The method is available for licensing from Yokohama City University.

Boettner, B. *SciBX* 6(29); doi:10.1038/scibx.2013.746
Published online Aug. 1, 2013

REFERENCES

1. Takebe, T. *et al. Nature*; published online July 3, 2013; doi:10.1038/nature12271
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2. Starzl, T.E. *et al. Am. J. Surg.* **129**, 587–590 (1975)
3. Booth, C. *et al. World J. Gastroenterol.* **18**, 6926–6934 (2012)
4. Wertheim, J.A. *et al. Curr. Opin. Organ Transplant.* **17**, 235–240 (2012)
5. Si-Tayeb, K. *et al. Hepatology* **51**, 297–305 (2010)
6. Espejel, S. *et al. J. Clin. Invest.* **120**, 3120–3126 (2010)
7. Soto-Gutierrez, A. *et al. Tissue Eng. Part C Methods* **17**, 677–686 (2011)

COMPANIES AND INSTITUTIONS MENTIONED

Hepregen Corp., Medford, Mass.
McGowan Institute for Regenerative Medicine, Pittsburgh, Pa.
Miromatrix Inc., Minneapolis, Minn.
Sekisui Medical Co. Ltd., Tokyo, Japan
The University of Edinburgh, Edinburgh, U.K.
University of Minnesota, Minneapolis, Minn.
University of Pittsburgh, Pittsburgh, Pa.
University of Pittsburgh Medical Center, Pittsburgh, Pa.
Wake Forest Institute for Regenerative Medicine, Winston-Salem, N.C.
Yokohama City University, Yokohama, Japan

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
Cancer				
Acute myelogenous leukemia (AML)	Integrin β_3 (GPIIIa; CD61)	Studies in mice suggest antagonizing CD61 could help treat AML. In a mouse xenograft model for AML, knockdown or deletion of *Cd61* slowed leukemia progression and increased survival compared with wild-type *Cd61* expression without affecting hematopoiesis. Next steps could include testing anti-CD61 mAbs in mouse models for AML. ***SciBX* 6(29); doi:10.1038/scibx.2013.747** **Published online Aug. 1, 2013**	Patent and licensing status undisclosed	Miller, P.G. *et al. Cancer Cell*; published online June 13, 2013; doi:10.1016/j.ccr.2013.05.004 **Contact:** Benjamin L. Ebert, Harvard Medical School, Boston, Mass. e-mail: bebert@partners.org
Brain cancer	Cytosolic branched chain amino-acid transaminase 1 (BCAT1); isocitrate dehydrogenase 1 (IDH1)	Patient tissue and mouse studies suggest inhibiting BCAT1 could be useful for treating gliomas with wild-type IDH1. In patient samples, BCAT1 was overexpressed in wild-type IDH1 gliomas and downregulated in mutant IDH1 gliomas. In a mouse xenograft model for wild-type IDH1 human glioma, small hairpin RNA against BCAT1 decreased tumor growth compared with nontargeting shRNA. Next steps could include identifying and evaluating small molecule BCAT1 inhibitors in gliomas with wild-type IDH1. ***SciBX* 6(29); doi:10.1038/scibx.2013.748** **Published online Aug. 1, 2013**	Patent and licensing status unavailable	Tönjes, M. *et al. Nat. Med.*; published online June 23, 2013; doi:10.1038/nm.3217 **Contact:** Bernhard Radlwimmer, German Cancer Research Center, Heidelberg, Germany e-mail: b.radlwimmer@dkfz-heidelberg.de
Breast cancer	Histone deacetylase 1 (HDAC1); HDAC6; estrogen receptor-α	Cell culture studies suggest bifunctional compounds inhibiting HDAC1, HDAC6 and estrogen receptors (ERs) could help treat ER^+ breast cancer. In human breast cancer cells, conjugates of the HDAC inhibitor Zolinza vorinostat with the generic ER antagonist tamoxifen showed more potent antiproliferative effects against ER^+ cells than ER^- cells. In breast cancer cell lines, the bifunctional compounds caused twofold more inhibition of growth than tamoxifen alone. Next steps include optimization of lead compounds and evaluating tumor-selective accumulation and efficacy in mouse models. Merck & Co. Inc. and Taiho Pharmaceutical Co. Ltd. market Zolinza to treat cutaneous T cell lymphoma (CTCL). ***SciBX* 6(29); doi:10.1038/scibx.2013.749** **Published online Aug. 1, 2013**	Patent status undisclosed; available for licensing from the Georgia Tech Research Corp.	Gryder, B.E. *et al. J. Med. Chem.*; published online June 20, 2013; doi:10.1021/jm400467w **Contact:** Adegboyega K. Oyelere, Georgia Institute of Technology, Atlanta, Georgia e-mail: aoyelere@gatech.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Breast cancer	Tissue factor	*In vitro* and mouse studies suggest inhibiting alternatively spliced tissue factor (asTF) could help treat breast cancer. In breast cancer cells implanted into mouse mammary fat pads, cells overexpressing asTF showed greater proliferation and anchorage-independent growth than cells overexpressing full-length TF. In mice implanted with asTF-overexpressing breast cancer cells, an asTF-targeting antibody inhibited proliferation. Next steps could include studies in additional mouse models. ***SciBX* 6(29); doi:10.1038/scibx.2013.750** **Published online Aug. 1, 2013**	Unpatented; licensing status unavailable	Kocatürk, B. *et al. Proc. Natl. Acad. Sci. USA*; published online June 25, 2013; doi:10.1073/pnas.1307100110 **Contact:** Henri H. Versteeg, Leiden University Medical Center, Leiden, the Netherlands e-mail: h.h.versteeg@lumc.nl
Cancer	Hyaluronan synthase 2 (HAS2); hyaluronidase 2 (HYAL2)	*In vitro* and rodent studies suggest extremely high molecular mass hyaluronan (HMM-HA) could help treat cancer. Cultured fibroblasts from the naked mole rat, a cancer-resistant species, synthesize HMM-HA and undergo growth arrest earlier and at lower cell densities than mouse and guinea pig fibroblasts that synthesize hyaluronan. In naked mole rat fibroblasts, an antibody that blocks the interaction between HMM-HA and its receptor inhibited early growth arrest. In mice injected with transformed naked mole fibroblasts, cells with decreased expression of HMM-HA by overexpression of Hyal2 or knockdown of Has2 increased tumor formation compared with cells with wild-type HMM-HA expression. Next steps include generating knock-in and transgenic mouse models that express naked mole rat Has2 and developing inhibitors against Hyal2. ***SciBX* 6(29); doi:10.1038/scibx.2013.751** **Published online Aug. 1, 2013**	Patent application filed; available for licensing	Tian, X. *et al. Nature*; published online June 19, 2013; doi:10.1038/nature12234 **Contact:** Andrei Seluanov, University of Rochester, Rochester, N.Y. e-mail: andrei.seluanov@rochester.edu **Contact:** Vera Gorbunova, same affiliation as above e-mail: vera.gorbunova@rochester.edu
Cancer	Phosducin-like 3 (PDCL3); VEGF receptor 2 (KDR/ Flk-1; VEGFR-2)	Cell culture studies suggest inhibiting PDCL3 could help treat VEGFR-2-dependent angiogenesis in tumors. In an aortic endothelial cell line, vector-mediated overexpression of *PDCL3* increased VEGFR-2 stability and VEGFR-2-dependent capillary tube formation compared with wild-type *PDCL3* expression. In a human umbilical vascular endothelial cell line, small interfering RNA–mediated knockdown of *PDCL3* decreased VEGFR-2 protein levels and VEGF-dependent capillary tube formation compared with no knockdown. In human embryonic kidney cells, coexpression of *VEGFR-2* and *PDCL3* increased cell proliferation compared with expression of either protein alone. Next steps include conducting *in vivo* tests of PDCL3 function and developing small molecule inhibitors of PDCL3. ***SciBX* 6(29); doi:10.1038/scibx.2013.752** **Published online Aug. 1, 2013**	Patent and licensing status undisclosed	Srinavasan, S. *et al. J. Biol. Chem.*; published online June 21, 2013; doi:10.1074/jbc.M113.473173 **Contact:** Nader Rahimi, Boston University Medical Campus, Boston, Mass. e-mail: nrahimi@bu.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer	Potassium channel KCa2.3 (KCNN3; SK3); transmembrane protein 142A (ORAI1; TMEM142A; CRACM1)	Mouse studies suggest inhibiting the interaction between the SK3 and ORAI1 channels could help prevent cancer metastasis. Mice grafted with *SK3*-deficient human breast cancer cells showed lower cancer cell migration and bone metastasis than those grafted with nondeficient cells. In cultured breast cancer cells and in mouse xenograft models, the lipid ohmline, which inhibits the localization of SK3 and ORAI1 on lipid rafts, decreased cell migration and bone metastasis compared with vehicle. Next steps include determining whether expression of SK3-ORAI1 complex in tumors correlates with risk of bone metastasis. ***SciBX* 6(29); doi:10.1038/scibx.2013.753** **Published online Aug. 1, 2013**	Reported compounds and screening method to identify anticancer compounds targeting SK3 patented; available for licensing	Chantôme, A. *et al. Cancer Res.*; published online June 17, 2013; doi:10.1158/0008-5472.CAN-12-4572 **Contact:** Christophe Vandier, University Francois Rabelais, Tours, France e-mail: christophe.vandier@univ-tours.fr
Cancer	Purinergic receptor P2Y G protein–coupled 2 (P2RY2; P2Y2)	*In vitro* and mouse studies suggest antagonizing the adenine nucleotide receptor P2Y2 could help prevent cancer metastasis. In cell cocultures, addition of platelets or platelet-derived adenine nucleotides increased tumor cell migration through an endothelial cell layer compared with no treatment. Knockout of *P2Y2* in the endothelial cells prevented tumor cell migration across the endothelial cell layer. In mice receiving subcutaneous or i.v. injection of murine melanoma or lung carcinoma cells, *P2y2*-deficient mice showed less tumor cell extravasation and metastasis than wild-type controls. Next steps include developing potent and specific P2Y2 receptor inhibitors. ***SciBX* 6(29); doi:10.1038/scibx.2013.754** **Published online Aug. 1, 2013**	Patent application filed; available for licensing	Schumacher, D. *et al. Cancer Cell*; published online June 27, 2013; doi:10.1016/j.ccr.2013.05.008 **Contact:** Stefan Offermanns, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany e-mail: stefan.offermanns@mpi-bn.mpg.de
Liver cancer	Deoxycholic acid	Mouse studies suggest decreasing deoxycholic acid production or deoxycholic acid–producing bacteria in the gut could help prevent obesity-associated hepatocellular carcinoma (HCC). In obese, HCC-prone mice, vancomycin decreased both serum levels of deoxycholic acid and tumor burden in the liver compared with vehicle control. Pharmacological inhibition of deoxycholic acid production also led to decreased tumor burden in the liver. Next steps include determining whether levels of deoxycholic acid or deoxycholic acid–producing bacteria could be used as biomarkers in obesity-associated cancer (*see* **Connecting the microbiome to obesity-associated cancers, page 1**). ***SciBX* 6(29); doi:10.1038/scibx.2013.755** **Published online Aug. 1, 2013**	Patent application filed; available for licensing and partnering	Yoshimoto, S. *et al. Nature*; published online June 26, 2013; doi:10.1038/nature12347 **Contact:** Eiji Hara, Japanese Foundation for Cancer Research, Tokyo, Japan e-mail: eiji.hara@jfcr.or.jp

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Neuroendocrine tumors	Histone deacetylase 10 (HDAC10)	Computational and cell culture studies suggest HDAC10 inhibitors could enhance chemotherapy in treating neuroblastoma. In a database of patients with advanced-stage neuroblastoma, high *HDAC10* expression correlated with poor overall survival. In neuroblastoma cells, *HDAC10*-targeting small hairpin RNA increased doxorubicin-mediated cell death compared with control shRNA. Next steps include targeting HDAC10 in animal tumor models. ***SciBX* 6(29); doi:10.1038/scibx.2013.756** **Published online Aug. 1, 2013**	Patented; available for licensing	Oehme, I. *et al. Proc. Natl. Acad. Sci. USA*; published online June 25, 2013; doi:10.1073/pnas.1300113110 **Contact:** Ina Oehme, German Cancer Research Center, Heidelberg, Germany e-mail: i.oehme@dkfz.de
Prostate cancer	Kallikrein-related peptidase 4 (KLK4)	Cell culture and mouse studies suggest inhibition of KLK4 could help treat prostate cancer. In an androgen-sensitive prostate cancer cell line, small hairpin RNA–mediated knockdown of *KLK4* decreased cell proliferation and increased apoptotic cell death compared with no knockdown. In mice, injection of *Klk4*-depleted prostate cancer cells resulted in slower tumor growth than injection of nondepleted cells. In a mouse xenograft model for prostate cancer, *Klk4* small interfering RNA increased tumor regression compared with control siRNA. Next steps include optimization of siRNA delivery and generation of small molecule KLK4 inhibitors. ***SciBX* 6(29); doi:10.1038/scibx.2013.757** **Published online Aug. 1, 2013**	Unpatented; unavailable for licensing	Jin, Y. *et al. Proc. Natl. Acad. Sci. USA*; published online June 24, 2013; doi:10.1073/pnas.1304318110 **Contact:** Fahri Saatcioglu, University of Oslo, Oslo, Norway e-mail: fahris@ibv.uio.no
Cardiovascular disease				
Heart failure	Not applicable	A zebrafish study suggests atrial cardiomyocytes can be converted to ventricular cardiomyocytes, which could be useful for treating heart failure. In zebrafish, chemically induced ventricular cardiomyocyte death resulted in proliferation and migration of atrial cardiomyocytes into ventricles. In the zebrafish, the migrating cardiomyocytes lost expression of atria-specific markers and gained expression of ventricle-specific markers, indicative of transdifferentiation, which led to recovered ventricular function. Next steps include generating ventricular cardiomyocytes *in vitro* from various cellular sources, including atrial cardiomyocytes, to rescue heart failure in mouse models. ***SciBX* 6(29); doi:10.1038/scibx.2013.758** **Published online Aug. 1, 2013**	Unpatented; licensing not applicable	Zhang, R. *et al. Nature*; published online June 19, 2013; doi:10.1038/nature12322 **Contact:** Neil C. Chi, University of California, San Diego, La Jolla, Calif. e-mail: nchi@ucsd.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Endocrine/metabolic disease				
Mucopolysaccharidosis	*N-sulfoglucosamine sulfohydrolase* (*SGSH*; *HNS*)	Animal studies suggest *SGSH* gene therapy could help treat mucopolysaccharidosis IIIA (MPS IIIA). In mouse models for MPS IIIA and in normal dogs, intracerebral injection of an adeno-associated virus serotype 9 (AAV9) vector encoding murine or canine *Sgsh* increased Sgsh activity in the brain compared with injection of empty vector. In the mouse models, the gene therapy decreased behavioral deficits and disease symptoms in peripheral organs and increased locomotor function and survival compared with empty vector. Ongoing studies include testing intracerebral delivery of an AAV9 vector–based gene therapy in animal models for MPS IIIB. Esteve S.A. has the AAV9 vector–based *SGSH* gene therapy in preclinical testing to treat MPS IIIA. Lysogene's SAF-301, an intraparenchymally administered AAV10 vector encoding human *SGSH* and *sulfatase modifying factor 1* (*SUMF1*), has completed Phase I/II testing to treat MPS IIIA. ***SciBX* 6(29); doi:10.1038/scibx.2013.759** **Published online Aug. 1, 2013**	Patented by the Autonomous University of Barcelona and Esteve; licensed to Esteve	Haurigot, V. *et al. J. Clin. Invest.*; published online July 1, 2013; doi:10.1172/JCI66778 **Contact:** Fátima Bosch, Autonomous University of Barcelona, Bellaterra, Spain e-mail: fatima.bosch@uab.es
Infectious disease				
Chagas disease; malaria; trypanosome	Not applicable	*In vitro* and mouse studies suggest *m*-terphenyl and dipyridylbenzene compounds could help treat protozoan infections. *In vitro* assays identified *m*-terphenyl and dipyridylbenzene analogs that inhibited the activity of *Trypanosoma brucei rhodesiense*, *T. cruzi* and *Plasmodium falciparum* at nanomolar IC_{50} values. In mouse models for trypanosome infection, several lead compounds increased relapse-free survival compared with the generic antiprotozoal drugs pentamidine and melarsoprol. Next steps could include testing the lead compounds in mouse models for malaria infection. Pentamidine is approved to treat pneumonia caused by *Pneumocystis carinii*, and melarsoprol is approved to treat human African trypanosomiasis. ***SciBX* 6(29); doi:10.1038/scibx.2013.760** **Published online Aug. 1, 2013**	Patent and licensing status unavailable	Patrick, D.A. *et al. J. Med. Chem.*; published online June 24, 2013; doi:10.1021/jm400508e **Contact:** Richard R. Tidwell, The University of North Carolina at Chapel Hill, Chapel Hill, N.C. e-mail: tidwell@med.unc.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Gram-negative bacterial infection	Capsular polysaccharide export protein (wza)	Cell culture studies identified a glycomimetic inhibitor of wza that could help treat Gram-negative bacterial infections. An *in vitro* screen identified an unnatural cyclic glycomimetic that could inhibit wza activity by blocking the protein's α-helix barrel. In a pathogenic strain of *Escherichia coli* cultured in human serum, the glycomimetic blocked the transport of a capsular polysaccharide and caused defects in the bacterial outer membrane, and it increased complement-mediated killing of bacteria compared with no treatment. Next steps include evaluating the glycomimetic in animal models. ***SciBX* 6(29); doi:10.1038/scibx.2013.761** **Published online Aug. 1, 2013**	Patent application filed; available for licensing from Isis Innovation Ltd. **Contact:** Mark Gostock, Isis Innovation Ltd., Oxford, U.K. e-mail: mark.gostock@isis.ox.ac.uk	Kong, L. *et al. Nat. Chem.*; published online June 30, 2013; doi:10.1038/nchem.1695 **Contact:** Hagen Bayley, University of Oxford Chemical Research Laboratory, Oxford, U.K. e-mail: hagan.bayley@chem.ox.ac.uk **Contact:** Benjamin G. Davis, same affiliation as above e-mail: ben.davis@chem.ox.ac.uk
Malaria	Not applicable	A chemically inactivated, whole-parasite vaccine could help treat malaria infection. Mice immunized with red blood cells containing a chemically inactivated rodent plasmodium parasite rapidly cleared a plasmodium infection, whereas mice immunized with plasmodium-infected control red blood cells did not. *In vitro*, *Plasmodium falciparum* was attenuated by a DNA-alkylating agent. Next steps include starting a clinical trial of a chemically attenuated, whole-cell malaria vaccine this year. ***SciBX* 6(29); doi:10.1038/scibx.2013.762** **Published online Aug. 1, 2013**	Patent applications filed; unavailable for licensing	Good, M.F. *et al. J. Clin. Invest.*; published online July 1, 2013; doi:10.1172/JCI66634 **Contact:** Michael F. Good, Griffith University, Gold Coast, Queensland, Australia e-mail: michael.good@griffith.edu.au
Malaria	*Plasmodium falciparum* multidrug resistance protein 1 (PfMDR1)	*In vitro* and cell culture studies identified a PfMDR1-targeting compound, ACT-213615, that could help treat malaria. In biochemical studies with *P. falciparum* cell lysates, ACT-213615 bound directly to PfMDR1. In *P. falciparum* cultures, ACT-213615 decreased proliferation at all asexual stages of the parasite cell cycle compared with an inactive control compound. Next steps could include optimizing the lead compound and testing it in models for malarial infection. Actelion Ltd. has an undisclosed molecule in Phase I testing for malaria. ***SciBX* 6(29); doi:10.1038/scibx.2013.763** **Published online Aug. 1, 2013**	Patented by Actelion; available for licensing	Brunner, R. *et al. J. Biol. Chem.*; published online June 10, 2013; doi:10.1074/jbc.M113.453159 **Contact:** Christoph Binkert, Actelion Ltd., Allschwil, Switzerland e-mail: christoph.binkert@ext.actelion.com

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Tuberculosis	*Mycobacterium tuberculosis* long-chain fatty acid–CoA ligase (fadD32)	*In vitro* and mouse studies identified fadD32 inhibitors that could help treat tuberculosis. 4,6-Diaryl-5,7-dimethyl coumarin derivatives potently and selectively killed *M. tuberculosis* strains at submicromolar concentrations by inhibiting fadD32, a component of the mycolic acid biosynthesis pathway. In mice infected with *M. tuberculosis*, intraperitoneal administration of the most potent coumarin derivative cleared bacterial burden from the lungs as effectively as isoniazid. Next steps could include testing the new compounds in additional preclinical models. Isoniazid is a generic tuberculosis therapeutic. ***SciBX* 6(29); doi:10.1038/scibx.2013.764** **Published online Aug. 1, 2013**	Patent and licensing status unavailable	Stanley, S.A. *et al. Proc. Natl. Acad. Sci. USA*; published online June 24, 2013; doi:10.1073/pnas.1302114110 **Contact:** Deborah T. Hung, Broad Institute of MIT and Harvard, Cambridge, Mass. e-mail: hung@molbio.mgh.harvard.edu
Neurology				
Pain	μ-Opioid receptor (OPRM1; MOR); metabotropic glutamate receptor subtype 5 (mGluR5; GRM5)	A mouse study suggests bivalent ligands with OPRM1 agonist and mGluR5 antagonist activity could help treat chronic inflammatory pain. In lipopolysaccharide (LPS)-pretreated mice, intrathecal administration of bivalent inhibitors yielded a more potent anti-nociceptive response in tail flick assays than administration of monovalent inhibitors without inducing acute tolerance. Next steps include generation of bivalent ligands for other heteromeric receptors. At least six companies have mGluR5 antagonists in Phase III or earlier development for neurology indications. ***SciBX* 6(29); doi:10.1038/scibx.2013.765** **Published online Aug. 1, 2013**	Series of bivalent ligands patented; licensing status unavailable	Akgün, E. *et al. Proc. Natl. Acad. Sci. USA*; published online June 24, 2013; doi:10.1073/pnas.1305461110 **Contact:** Philip S. Portoghese, University of Minnesota, Minneapolis, Minn. e-mail: porto001@maroon.tc.umn.edu
Various				
Atherosclerosis; Alzheimer's disease (AD); diabetes; inflammation	CD36 (GPIV)	*In vitro* and mouse studies suggest inhibiting CD36 could help suppress inflammation in atherosclerosis, AD and type 2 diabetes. Macrophages convert soluble forms of low-density lipoprotein (LDL) into cholesterol crystals and β-amyloid (Aβ) into amyloid deposits, which can provoke inflammatory responses in the vasculature, brain and pancreas. In mice and mouse macrophages deficient in Cd36, the inflammatory response was lower than that in nondeficient controls. In a mouse model for atherosclerosis, an antisense oligonucleotide against CD36 decreased inflammatory reactions and plaque formation compared with a control oligonucleotide. Next steps include testing CD36 antisense strategies in mouse models for other diseases that involve plaque formation such as AD. Isis Pharmaceuticals Inc. provided the CD36 antisense oligonucleotide. Arteria S.A. has a CD36 inhibitor in preclinical development for dyslipidemia. ***SciBX* 6(29); doi:10.1038/scibx.2013.766** **Published online Aug. 1, 2013**	Findings unpatented; licensing status not applicable	Sheedy, F.J. *et al. Nat. Immunol.*; published online June 30, 2013; doi:10.1038/ni.2639 **Contact:** Kathryn J. Moore, New York University Langone Medical Center, New York, N.Y. e-mail: kathryn.moore@nyumc.org

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cystic fibrosis (CF); muscular dystrophy	Ribosome	*In vitro* studies have questioned the mechanism of action for ataluren (PTC124), which is in clinical development to treat CF and Duchenne muscular dystrophy (DMD). The proposed mechanism of action for ataluren is to induce readthrough of premature termination codons (PTCs) by ribosomes, thus increasing protein expression in genetic disorders for which PTCs cause disease. In a series of *in vitro* assays to measure PTC readthrough, ataluren had no effect, whereas the aminoglycoside antibiotic geneticin had a dose-dependent response. Next steps could include studies designed to further explore the mechanism of action for drugs thought to induce PTCs *in vivo*. PTC Therapeutics Inc. has ataluren in Phase III testing to treat nonsense mutation DMD. Last year, the EMA accepted for review an MAA seeking conditional approval of ataluren to treat nonsense mutation DMD. The product also has completed a Phase III trial to treat nonsense mutation CF, with a confirmatory Phase III trial in the indication expected to start next year. ***SciBX* 6(29); doi:10.1038/scibx.2013.767** **Published online Aug. 1, 2013**	Patent and licensing status not applicable	McElroy, S.P. *et al. PLoS Biol.*; published online June 25, 2013; doi:10.1371/journal.pbio.1001593 **Contact:** Stuart P. McElroy, University of Dundee, Dundee, U.K. e-mail: s.mcelroy@dundee.ac.uk

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
Assays & screens			
Quantification of clustered, regularly interspaced short palindromic repeats (CRISPR)-based editing system specificity	Quantification of off-target mutagenesis in CRISPR-based genome editing systems could help evaluate CRISPR systems for potential therapeutic applications and guide the development of CRISPR systems with increased accuracy. CRISPR systems use a small guide RNA, which pairs with a target DNA sequence and the CRISPR-associated protein (Cas9) to excise target DNA. The system recently has been used to rapidly engineer mice and cell lines carrying multiple mutations. A human cell–based GFP assay was used to quantify rates of off-target mutagenesis and found a range of 5.6%–125% off-target activity. Next steps include whole-genome mapping of off-target sites. ***SciBX* 6(29); doi:10.1038/scibx.2013.768** **Published online Aug. 1, 2013**	Patent and licensing status not applicable	Fu, Y. *et al. Nat. Biotechnol.*; published online June 23, 2013; doi:10.1038/nbt.2623 **Contact:** Jeffry D. Sander, Massachusetts General Hospital, Charlestown, Mass. e-mail: jsander@partners.org **Contact:** J. Keith Joung, same affiliation as above e-mail: jjoung@partners.org
Computational models			
Computational model for predicting P glycoprotein (MDR1; ABCB1; P-gp; CD243) substrates	A computational model to predict whether a compound may be exported by P-gp could help identify cancer therapeutics that avoid P-gp-mediated resistance. Cytotoxicity data from about 13,000 compounds screened against a panel of 60 human cancer cell lines was used to predict 448 compounds as P-gp substrates and 486 compounds as nonsubstrates. An analysis of these compounds led to the development of a computational model that classified compounds as P-gp substrates or nonsubstrates with 86% accuracy and 82% precision in an independent test set. Next steps include using the model to predict P-gp substrate status for therapeutic candidates and members of chemical libraries. ***SciBX* 6(29); doi:10.1038/scibx.2013.769** **Published online Aug. 1, 2013**	Unpatented; use of predictive model freely available at http://pgp.biozyne.com; advanced features available for licensing	Levatić, J. *et al. J. Med. Chem.*; published online June 17, 2013; doi:10.1021/jm400328s **Contact:** Fran Supek, BioZyne Ltd., Zagreb, Croatia e-mail: fran.supek@irb.hr
Disease models			
Mouse model for prostate cancer driven by v-ets erythroblastosis virus E26 oncogene homolog (ERG) translocations	Mice with prostate-specific Erg expression could be used as a model to study prostate cancer pathogenesis. Translocations of ETS transcription factors including ERG are common in prostate cancer but have been difficult to model in mice. To develop a mouse model to study the role of ERG in prostate cancer, prostate-specific *Erg* overexpression was combined with homozygous loss of *Pten* (*Mmac1*; *Tep1*), which led to the development of invasive prostate adenocarcinomas in 80% of the animals at 6 months. In *Pten*-deficient prostates, *Erg* overexpression increased the expression of androgen receptor–regulated genes compared with wild-type *Erg* expression. Next steps could include using the model to identify new therapeutic targets. ***SciBX* 6(29); doi:10.1038/scibx.2013.770** **Published online Aug. 1, 2013**	Patent and licensing status unavailable	Chen, Y. *et al. Nat. Med.*; published online June 30, 2013; doi:10.1038/nm.3216 **Contact:** Charles L. Sawyers, Memorial Sloan-Kettering Cancer Center, New York, N.Y. e-mail: sawyersc@mskcc.org **Contact:** Yu Chen, same affiliation as above e-mail: cheny1@mskcc.org
Mouse models for choroidal neovascularization (CNV) and age-related macular degeneration (AMD)	Mouse models for CNV could help identify therapeutics to treat AMD. Abnormal vascularization of the retina or choroid can give rise to AMD. VEGF receptor 1 (FLT1; VEGFR-1) inhibits VEGF-A to prevent abnormal angiogenesis. In mice, decreasing Flt1 activity with an antibody or small hairpin RNA–mediated gene knockdown caused CNV. In mice, induced loss of Flt1 expression in two different layers of the retina resulted in CNV. Ongoing studies include testing the therapeutic effect of restoring FLT1 expression in the CNV models. ***SciBX* 6(29); doi:10.1038/scibx.2013.771** **Published online Aug. 1, 2013**	Models unpatented; licensing status unavailable	Luo, L. *et al. eLife*; published online June 18, 2013; doi:10.7554/eLife.00324 **Contact:** Balamurali K. Ambati, The University of Utah, Salt Lake City, Utah e-mail: bambati@gmail.com **Contact:** Ling Luo, same affiliation as above e-mail: ling.luoling1208@gmail.com

This week in techniques

Approach	Summary	Licensing status	Publication and contact information
Drug platforms			
Human bone marrow–specific extracellular matrix (ECM) substrates	Human bone marrow–specific ECM substrates could be used to culture stem cells for therapeutic applications. Bone marrow–derived mesenchymal stem cells (MSCs) were cultured under conditions that induced the production of osteogenic bone marrow ECM or collagen-rich ECM, and then the cells were removed using a decellularization protocol. *In vitro*, MSCs and hematopoietic stem and progenitor cells (HSPCs) showed greater proliferation when cultured on the MSC-derived ECM substrates than when cultured on conventional tissue culture plastic. HSPCs generated on the ECM substrates showed better engraftment in mice than those seeded on control scaffolds. Next steps include using immortalized human MSCs and other fibroblast-like cells to generate decellularized matrices and standardizing the matrix production procedure for clinical use. ***SciBX* 6(29); doi:10.1038/scibx.2013.772** **Published online Aug. 1, 2013**	Patent application filed; licensing details available from the Leibniz Institute of Polymer Research Dresden	Prewitz, M.C. *et al. Nat. Methods*; published online June 23, 2013; doi:10.1038/nmeth.2523 **Contact:** Carsten Werner, Max Bergmann Center of Biomaterials Dresden, Dresden, Germany e-mail: carsten.werner@tu-dresden.de
Monoacyl lipopeptide agonist of toll-like receptor 2 (TLR2) as a vaccine adjuvant	A lipopeptide agonist of TLR2 could boost the efficacy of vaccines against infectious diseases. Chemical synthesis, SAR and *in vitro* testing of monoacyl lipopeptides identified a water soluble, nanomolar agonist of TLR2. In rabbits immunized with a vaccine based on the lactalbumin-α (Lalba) antigen or a diphtheria toxin antigen, coimmunization with the lead compound increased antigen-specific IgG levels in serum compared with coimmunization using vehicle. Next steps include testing the lead compound as an adjuvant to influenza vaccines in animals. ***SciBX* 6(29); doi:10.1038/scibx.2013.773** **Published online Aug. 1, 2013**	Patented; available for licensing	Salunke, D.B. *et al. J. Med. Chem.*; published online June 24, 2013; doi:10.1021/jm400620g **Contact:** Sunil A. David, The University of Kansas, Lawrence, Kan. e-mail: sdavid@ku.edu
Platform for maturation of human pluripotent stem cell–derived cardiomyocytes	A platform for generating mature cardiomyocytes from human pluripotent stem cells could aid the development of improved models of cardiovascular disease. Human pluripotent stem cell–derived cardiomyocytes normally have a fetal phenotype rather than an adult phenotype. To induce an adult phenotype, human pluripotent stem cell–derived cardiomyocytes were arranged in a wire pattern within a 3D culture system and then subjected to electrical stimulation. In culture, cardiomyocytes subjected to electrical stimulation showed more adult-like qualities than nonstimulated cardiomyocytes. Next steps include using the generated cardiomyocytes in drug discovery and validation studies and incorporating both electrical and mechanical stimulation to help promote terminal differentiation of the cells. ***SciBX* 6(29); doi:10.1038/scibx.2013.774** **Published online Aug. 1, 2013**	Patent application filed; available for licensing and collaboration	Nunes, S.S. *et al. Nat. Methods*; published online June 23, 2013; doi:10.1038/nmeth.2524 **Contact:** Milica Radisic, University of Toronto, Toronto, Ontario, Canada e-mail: m.radisic@utoronto.ca
Self-assembling, osteogenic, polymer-based coating to prevent joint implant failure	Rodent studies suggest a self-assembling, osteogenic, polymer-based coating could help prevent joint implant failure. The multilayer polymer coating is less than 2 μm thick and consists of a permanent osteoconductive hydroxyapatite base layer underneath hydrolytically degradable osteoinductive poly(β-amino ester) layers that slowly release bone morphogenetic protein 2 (BMP2). In a rat model for implant integration, implants that used the multilayer polymer coating showed better integration with host bone and greater tensile strength at the bone-implant interface than implants stabilized with conventional bone cement. In the model, implants with the polymer coating showed long-term stable fixation to host bone and no fracturing at the bone-implant interface as measured out to 18 months. Next steps include evaluating implants that use the coating in large animal models. ***SciBX* 6(29); doi:10.1038/scibx.2013.775** **Published online Aug. 1, 2013**	Patent application filed; available for licensing	Shah, N.J. *et al. Sci. Transl. Med.*; published online June 26, 2013; doi:10.1126/scitranslmed.3005576 **Contact:** Paula T. Hammond, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: hammond@mit.edu

This week in techniques

Approach	Summary	Licensing status	Publication and contact information
Markers			
Androgen receptor mutations that convey resistance to second-generation anti–androgen receptor compounds	Studies in cell culture and in patient samples identified drug resistance mutations that could help guide prostate cancer treatment. In cells, chronic exposure to the anti–androgen receptor compounds Xtandi enzalutamide or ARN-509 resulted in the acquisition of the F876L androgen receptor mutation. In cells with the F976L mutation, the drugs acted as agonists, whereas in wild-type cells the drugs acted as antagonists and inhibited cell proliferation. In circulating tumor DNA obtained from patients' plasma, the mutation was identified after treatment with ARN-509. Next steps include determining the frequency of the mutation in patients receiving second-generation anti–androgen receptor compounds. Xtandi enzalutamide is marketed by Astellas Pharma Inc. and Medivation Inc. for castration-resistant prostate cancer. ARN-509 from Aragon Pharmaceuticals Inc. is in Phase II testing. ***SciBX* 6(29); doi:10.1038/scibx.2013.776** **Published online Aug. 1, 2013**	Patent application filed; licensing status undisclosed	Joseph, J.D. *et al. Cancer Discov.*; published online June 18, 2013; doi:10.1158/2159-8290.CD-13-0226 **Contact:** James D. Joseph, Aragon Pharmaceuticals Inc., San Diego, Calif. e-mail: jjoseph@aragonpharm.com
Galectin-1 (LGALS1) as a prognostic marker of early onset preeclampsia	Monitoring levels of serum LGALS1 could help predict risk of severe preeclampsia. In 24 pregnant women, circulating levels of LGALS1 were lower during the second trimester in women who developed preeclampsia than levels in women who did not. In pregnant mice, *Lgals1* knockout or an LGALS1 inhibitor administered in early pregnancy increased blood pressure, proteinuria and other symptoms seen in human preeclampsia compared with no knockout or no treatment, respectively. Ongoing work includes validating serum LGALS1 as a prognostic marker of preeclampsia in a large cohort of pregnant women. ***SciBX* 6(29); doi:10.1038/scibx.2013.777** **Published online Aug. 1, 2013**	Patent and licensing status undisclosed	Freitag, N. *et al. Proc. Natl. Acad. Sci. USA*; published online June 24, 2013; doi:10.1073/pnas.1303707110 **Contact:** Sandra M. Blois, Charité–University Hospital Berlin, Berlin, Germany e-mail: sandra.blois@charite.de

Company and institution index

Target and compound index