Single Cell Gene Expression Studies Reveal Kidney Clues

By Bridget M. Kuehn

A new genetic technique called single cell genetic sequencing is helping to reveal new insights about the cells that make up the kidney—insights that are essential to understanding what goes wrong in kidney disease and how it might be reversed.

Many types of cells make up the kidney, each with a distinct role in kidney health, making it a challenge for scientists to study. Traditionally, scientists have tried to distinguish these various cell types by their location in the kidney and appearance under a microscope, according to Katalin Susztak, MD, PhD, a professor of medicine at the Perelman School of Medicine at the University of Pennsylvania. But rapid advances in genetic techniques are giving scientists new tools for studying these cells.

Whole genome sequencing studies have allowed scientists to document an individual’s entire genetic blueprint. Now the technology has advanced to allow scientists to look at which genes are turned on or off in individual cells.

“Every cell in your body has a full complement of DNA coding for approximately 21,000 proteins,” said Mark Knepper, MD, PhD, a senior investigator at the National Heart, Lung and Blood Institute. “However, each cell expresses only around 6000 to 8000 of these genes.”

Single cell RNA sequencing allows scientists to determine which 6000 to 8000 genes are turned on in each cell, which “provides a road map” for studying specific cell types, Knepper said.

“The single cell RNA sequencing approach and other next generation sequencing methods will provide essential information at a basic science level that will ultimately result in a better understanding of many renal diseases,” Knepper said.

Emerging insights

A groundbreaking study by Susztak and colleagues using single cell sequencing identified several new types of cells in the kidney, and showed that some cells in the kidney can transition back and forth between two cell types to help the kidney adapt to changing conditions.

In their study, published in Science, Susztak and her colleagues took one kidney from seven different male mice and used droplet-based single cell RNA sequencing to analyze the gene expression in the more than 43,745 cells in each kidney. They used a special machine to connect each cell with a bead that is able to capture all the genes that are active in that cell. Then, they sorted the cells based on these genes. This method is far cheaper than previous RNA sequencing methods, costing just 10 to 20 cents per sample, Susztak said, compared with older tech-
Changes in MOC

Continued from page 1

called the Vision Initiative commission, with the task “to pro-
vide a set of recommendations about the future of continuing board certification for consideration by ABMS.”

Rebellion brews at AMA

The movement for change has been building for years, ac-
cording to Donald J. Palmasino Jr., JD, executive director and CEO of the Medical Society of Georgia, which success-
fully pushed for MOC-limiting legislation last year.

“The physicians had reached their limits with the high-
stakes exam, and they felt frustrated by the fact that nobody was really listening to them. They had complained to their boards and their boards were not responding,” Palmasino said. “The physicians themselves got active and got their mes-
sage across, and the boards are responding now. They under-
stand that there are some challenges and they are trying to
fix them.”

Palmasino said that one turning point came at last year’s American Medical Association (AMA) meeting when the
CEOs of the American College of Obstetricians and Gy-

niquesthatcouldcost$300forsinglesample.

“Because the cost is much lower, we can actually se-
quence a large number of cells,” sheexplained.

Another advantage of this approach is that it provides an “unbiased” way of grouping cells, she said. The ap-
proachclassifiescellsonlyonthegeometricinformation be-
ing used in each cell instead of more superficial physical cell characteristics that might be shared by multiple types of cells. This allowed the researchers to discover that 1 of
the 3 types of cells previously identified in the collecting
duct of the kidney are actually just cells in transition from
one type to another. These results reinforce findings from
a previous study by Knepper and colleagues that had sug-
ggested a transitional cell type in the collecting duct.

“Both of us found hybrid cells that express markers of
both principal cells and intercalated cells,” Knepper said.
“Thissfindingadddsitadditional evidenceto ‘fate mapping’ studies that principal cells may convert into interca-
lated cells. This is currently a hot area of research.”

Scientists know that the collecting duct and its cells help balance salt, water, and acid–base levels in the body, according to Knepper. To keep up with changing de-
mands, it appears the cells may be able to transition from
being principal cells that transport water, sodium, and
potassium to intercalated cells that regulate acid–base bal-
ance by transporting hydrogen ions.

“We think in healthy adult kidneys this type of inter-
conversion happens on a regular basis to kind of balance the
water and acid, but this interconversion also hap-
pens more profoundly in [kidney disease] where the kid-
nneys might need to focus on water balance,” said Rojesh Shrestha, BS, a research specialist in Suzstrak’s laboratory.

Both Suzstrak and Knepper’s studies also found that
most principal cells in the collecting duct expressed the
Noto12 gene, and that the gene for its receptor is expressed
mostly by intercalated cells. This may help “explain how
principal cells and intercalated cells are able to ‘talk’ with
one another,” said Knepper. It may also have clinical im-
lications, noted Suzstrak, who explained that it might be possible to use treatments that manipulate these messages to intervene in diseases where the acid–base balance has
gone awry.

The insights were just part of a huge amount of data

generated in the study. Suzstrak and colleagues also showed
that the genes for specific kidney diseases were expressed
by just one type of cell. For example, genes linked with
high and low blood pressure were traced to one type of
cell. This insight may help scientists trying to pinpoint the
cause of certain diseases. Suzstrak suggested this likely
means that there is a very clear division of labor among
kidney cells, and if one type of cell is not working prop-
perly, for example because of a gene mutation, the others
need not pick up the slack.

The next step for Suzstrak’s research will be to start cata-
logue single gene expression in kidneys affected by dis-
ease to understand how gene expression changes. In the
meanwhile, she hopes the data from her current study will
help fuel other researchers’ work.

“We generated the periodic table for the kidney, so now we know all the elements in the kidney are,” she
said. “All the researchers who study kidney physiology or
kidney homeostasis will be able to put the elements to-
gether and understand disease development, and how the
kidney works under (healthy) conditions.”

Clinical implications

Already some groups have begun to apply single cell
sequencing techniques to samples taken from pa-
tients with kidney disease. For example, a network of
researchers from the Accelerating Medicines Part-
nership studying rheumatoid arthritis and systemic
lupus erythematosus recently used single cell gene
sequencing to analyze 16 kidney and 12 skin tissue
samples taken from patients with lupus nephritis dur-
ing routine care. The National Institutes of Health,
nonprofit groups, and industry are jointly funding the
network with the aim of accelerating the develop-
ment of new treatments.

The study provided a proof of concept that single cell
RNA sequencing might reveal useful information from
clinical samples. From just several millimeters of kidney
issue, the investigators were able to glean important infor-
mation that added on to earlier work with standard light
and electron microscopy studies of biopsies. In addition
to providing information that might help classify patients,
the study suggested that the sequencing data might also
hitch at a patient’s prognosis 6 months later.

“We’re getting a remarkable amount of information
which has direct clinical relevance,” said Chaim Putter-
man, MD, chief of the division of rheumatology at Albert
Einstein College of Medicine and Montefiore Medical
Center, who was the co-principal investigator, along with
Jill P. Buyon, MD, director of the division of rheuma-
tology at New York University School of Medicine, and
Thomas Tischel, PhD, head of the Laboratory for RNA
Molecular Biology at Rockefeller University in New York.

Putterman said that if other larger studies confirm the
potential prognostic value of single cell RNA sequencing,
it might encourage physicians to intensify initial treat-
ments for patients exhibiting molecular predictors of a
more aggressive disease.

The researchers also did single cell sequencing on skin
cells collected from patients with lupus to see if it might
provide useful information about the progression of the
disease. Putterman explained that kidney biopsies are
critical to assessing patients with lupus nephritis, but its
invasive nature together with the potential risks associated
with the procedure limit how many times it can be re-
peated. Skin cells could be more easily and safely collected
over time. The study showed that some of the same lupus-
linked genetic changes occurring in kidney cells may also
be seen in skin cells.

“If we can use the skin to reflect what’s happening in
the kidney, that would be a major advance forward,” Put-
terman said.

While single cell sequencing is an enormously promis-
ting technique and will likely lead to many new insights in
nephrology research, Knepper was cautious in his assess-
ment of the clinical potential of the technique. He noted
there are still many practical issues that would have to be
resolved before such technology could be used in the clin-
ic. For example, it’s difficult to apply to glomerular cells,
although some groups are working to solve this problem,
hesaid. It’s also not clear whether the process itself might
alter gene expression in the cells or if the genes expressed
by cells in isolation are the same ones expressed by cells in
the kidney that may be interacting with neighboring
cell types.

“That kind of thing limits the potential direct clini-
cal use,” Knepper said. “This is predominantly a research
method. With present technology, I don’t see this being
applied directly to a patient admitted to the hospital. But
the information the technique provides is marvelous and
unique.”


June 2018 | ASN Kidney News | 3

Continued on page 4
Changes in MOC

Continued from page 3

February 2019.

Some specialty society leaders at the De- cember meeting expressed suspicion that the commission could be a delaying tactic. But the Medical Society of Georgia's Palm iano—who was selected to be a member of the commission and attended its first meet- ing—said he is "cautiously optimistic that the concerns of the physicians are going to be addressed."

Checking in with nephrology

ABMS has said that all its boards are already implementing changes to "make their pro- grams more convenient, supportive, rel- evant, and cost-effective," and in this includes ABIM, which is introducing an option to replace the 10-year exam. Internal medicine and nephrology will be the first specialty and subspecialty to be offered this shorter test option, called the Knowledge Check-In, beginning in 2018.

The Knowledge Check-In can be taken every two years (can be taken at home, work, or a test center; is an open book test; using UpToDate as the permitted reference; and is offered four to six times a year. It is much shorter than the 10-year test, lasting three hours at most. A failed exam will not lead to loss of certification. Physicians will be able to take the test again two years later.

"The two-year Knowledge Check-In should be lower stress and certainly lower stakes, because you cannot lose your board certification by not doing well on one of these," Berne said. He said that the nephrol- ogy Knowledge Check-In has been working on adjusting its exam blueprint and honing the relevance of its questions for years. "Nephrology is actu- ally fortunate … to be the first subspecialty to be included in the Knowledge Check-In. It is because we had such a good exam pool, both in terms of quality and numbers of questions that could be rolled into this new format."

But the approach is still a summative, pass-or-fail test rather than a formative pro- cess more aimed at learning, according to Charles Cutler, MD, an internist and former president of the Pennsylvania Medical Soci- ety, who has been a critic of ABMS' MOC system. Cutler has also been appointed to the Vision Initiative commission.

Anesthesiologists lead the way

Cutler and other reformers cite the American Board of Anesthesiology for suc- cessfully introducing a formative process by dropping its every-10-years recertification test and replacing it with regular online tests and learning modules called the MOCA (Maintenance of Certification in Anesthesi- ology) Minute. "MOCA Minute allows you to continuously assess your knowledge, fill knowledge gaps and demonstrate your pro- ficiency," according to the board's website. The process is ongoing, with participants re- quired to answer 30 questions each quarter of the year.

"The American Board of Anesthesiology polled their diplomats and asked whether they like this system or the test. Some 80 or 85% of the doctors said, 'We like this new system. You are making us better with this," Cutler said.

Jim Soo Kim, ASN senior director of edu- cation, agrees that the Knowledge Check-In in its current form is a high-stakes, summa- tive approach that does not fully address ASN's concerns. ABIM is working with three of the larger specialty societies to develop alter- natives to the summative exam, but has not yet offered the same opportunity to smaller societies.

Cutler said he has been told by the lead- ership of other boards that they are "moving away from a summative process and going to a sequential formative process of identify- ing gaps in physicians' knowledge and filling in those gaps."

Kim also noted that many nephrologists have a specific focus, such as interventional nephrology, but the nephrology MOC ex- perts know that developing MOC for subspecialties will mean many questions not relevant to their prac- tices. In contrast, cardiologists, for example, can choose among exams focusing on five subspecialties. "We've been told that ABIM is open to exploring modularity in the fu- ture," she said.

Kim said that although the test will be open-book, UpToDate will be the only resource currently, despite the fact that spe- cialty societies offer resources such as ASN's Kidney Self-Assessment Program (KSAP). ABIM has indicated that this limitation has to do with technical issues and costs, but is open to adding more resources in the future.

Legislative efforts continue

Many state medical societies and legislatures remain dissatisfied with the pace of change. According to a compilation from ABMS, 17 bills introduced or pending in state legisla- tures prohibit the state licensing board from requiring MOC for licensure, 16 prohibit use of MOC by health plans for reimburse- ment, and 15 prohibit requiring physicians to participate in MOC for hospital privi- leges, credentialing, reimbursement, and employment. The great majority of this leg- islation died or is languishing in committees. ABMS has put resources into pressuring the legislation on the grounds that it under- mines the professional commitment to self-regulation, the standards for specialty certification, and public confidence in ABMS board certification.

Even proponents of the legislation acknowledge their hesitation about involving the government in physician self-regulation. Frank McDonald Jr., MD, MBA, a neu- rologist who is president of the Medical As- sociation of Georgia, said that it is a "slippery slope" to get the state government involved in physicians' affairs, but the association turned to the legislature because the mem- bership was "very frustrated" by the specialty boards adding requirements to MOC with- out listening to physicians' concerns.

"Everywhere I go, I talk about what the Medical Association of Georgia is doing," CMS Palmisano said. "This is one issue that unites physicians across the board. Every- body in the country is feeling the same way, that physicians weren't being listened to. Even the ones that have had a good experi- ence with their board and their MOC still get concerned that it could maybe go in a bad direction at one point. They want to make sure that doesn't happen."