Battling Kidney Transplant Rejection: Miniscule RNAs with Major Potential

Kidney transplants have been remarkably successful, but over the long term a substantial proportion of kidneys fail. The main reason for this failure includes acute and chronic rejection and other life-threatening complications such as infection and malignancy. For the last several years, Thangamani Muthukumar, MD, a member of the Division of Nephrology at NewYork-Presbyterian/Weill Cornell Medical Center and Associate Program Director (Research) of the Nephrology Fellowship Program, has been studying infinitesimally tiny yet apparently crucial players in an immunological war against a lifesaving organ transplant. The subject of the ongoing work of Dr. Muthukumar and his Weill Cornell colleagues is the role of microRNA (miRNA) – specifically purposed relatives of DNA and messenger RNA – as effective markers in detecting and as agents for controlling organ rejection processes.

Renal Transplantation: The Good, the Bad, and Hope for the Future

Kidney transplantation is the best treatment for patients with end-stage renal disease. However, there are many challenges faced by renal transplant specialists, including the shortage of available kidneys; improving organ allocation; increasing long-term survival; and preventing rejection. Transplant nephrology specialists in the Renal and Pancreatic Transplantation Program at NewYork-Presbyterian/Columbia University Medical Center face these issues daily. Fundamentally, their mission is to find more kidneys for transplantation and to get them to last longer. They take a multifaceted approach:

• First, they analyze how deceased donor kidneys are actually used for transplantation, and how this scarce resource can be better used.
• Second, they are developing ways to improve the outcomes of patients who receive transplants, so that transplanted kidneys last as long as possible and fewer people need to get a second transplant, currently 15 percent of those on the waitlist. This involves the testing of new immunosuppressive and other medications that will lead to better functioning transplants with fewer rejections and fewer drug side effects.
• Third, on the genetic level they are developing better tools to understand and predict who may be at risk for rejection.
• Finally, they are about to begin studies of tolerance, that is, creating a state in the transplant recipient in which all immunosuppressants can be permanently discontinued within a few months after transplantation without rejection. This will avoid all of the potential problems of long-term immunosuppression and eliminate the need for second transplants.
in the laboratory of my mentor Dr. Suthanthiran in an independent set of human kidney biopsies,” says Dr. Muthukumar.

“Sequencing is a powerful tool that provides an unbiased profiling of microRNAs. It allows us to catalog the universe of microRNAs,” says Dr. Muthukumar. “The combination of sequencing technique for biomarker discovery and polymerase chain reaction assay for quantification of targeted microRNAs is a powerful strategy to bring state-of-the-art technology from the research laboratory to the clinic for the benefit of our patients.” This study also provided preliminary evidence for an association between miRNA abundance level in the transplant kidney and its future function and survival.

Dr. Muthukumar’s goal is to determine the microRNA profile of every condition that affects the transplanted kidney. “We need to create a microRNA atlas of the transplant kidney,” he says. “There are more than a thousand human miRNAs that have been identified, and probably many more yet to be identified.” Since the publication of their seminal work in 2012, Dr. Muthukumar and his colleagues have continued to explore the potential of their findings with the objective of using microRNAs as biomarkers and to better understand the mechanisms of transplant rejection. Towards this goal, they are studying microRNA profiles in urine and blood samples of kidney transplants and other types of transplants, such as liver transplants, as well.

**Targeted Profiling of Messenger RNA in Urine Cells: A Simple Urine Test to Identify the Causes of Sudden Failure of the Transplant Kidney**

Continuing the pioneering work on messenger RNAs in Dr. Suthanthiran’s laboratory, Dr. Muthukumar led the research team that recently developed urinary cell messenger RNA-based molecular tests to distinguish the common causes of sudden failure of the transplant kidney. “At present, physicians have to do an invasive needle biopsy of the kidney to distinguish the common causes of sudden failure of the transplant kidney. It is important to differentiate among the different causes, as treatment is different for each. Our urine test is accurate to distinguish among the common causes and hence obviates the need for a needle biopsy,” says Dr. Muthukumar.

**Forecasting Kidney Allograft Failure**

More recently, the researchers reported on a study of 92 kidney transplant patients with transplant glomerulopathy – an important cause of late transplant failure – to develop and validate a prognostic score card based on the risk factors for the failure of the transplant kidney within five years of diagnosis of the disease. “Development of such a risk score model is important and may provide, for patients and physicians alike, informative insight about prognosis and treatment and eventually may help improve outcomes,” says Dr. Muthukumar.

In this largest cohort reported to date of kidney transplant recipients with transplant glomerulopathy, the researchers generated a prognostic index using sophisticated statistical techniques and, based on the score, categorized patients into risk groups for failure of the transplant kidney. The performance of the statistical model was verified in an independent external cohort of patients with the same diagnosis. “The risk factors that we identified were a novel combination of risk factors for chronic kidney disease, and the novelty lay in the successful development and application of the risk model to predict the most important cause of late failure of the kidney transplant,” says Dr. Muthukumar.

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With continuing support from the National Institutes of Health, Dr. Muthukumar believes that understanding the molecular basis of rejection, especially the role of the tiny microRNAs, will help to design personalized strategies to identify, treat, and eventually stop the transplant kidneys from failing. Dr. Muthukumar’s concentration on molecules so small is producing great opportunities for improving outcomes following kidney transplantation.

Research Update: Building on Pioneering Work in Predicting Kidney Transplant Rejection

In April 2014, a pioneering three-gene signature test for predicting kidney transplant rejection discovered by Manikkam Suthanthiran, MD, Chief of Nephrology and Hypertension at NewYork-Presbyterian/Weill Cornell, and his research team, was selected by the Clinical Research Forum as one of the Top 10 Outstanding Clinical Research Achievements in the United States.

In a new study, published online June 5, 2015, and expected in the February 2016 print issue of the Journal of the American Society of Nephrology, Dr. Suthanthiran and Karsten Suhre, PhD, from Weill Cornell Medical College in Qatar, examined whether the three-gene signature test was more effective in detecting kidney rejection than a new test that searched urine for the small-molecule byproducts of metabolism, called metabolites. The research team analyzed 1,516 samples from 241 kidney transplant recipients and found that the three-gene signature test performed better than the metabolite scan.

“Very interestingly, when the investigators combined both the three-gene signature and metabolite information, they discovered that the combined measures were more powerful than either one alone. This discovery could eventually replace invasive needle biopsies as the gold-standard diagnostic test for the condition, the investigators say, and could lead to earlier detection, perhaps even weeks or months before patients show any symptoms. “When fully developed, this new test of the combination of the three-gene signature and metabolite profile has the potential to help us manage transplanted kidneys more effectively and to significantly reduce the number of biopsies performed to diagnose rejection,” says Dr. Suthanthiran, senior author of the study. “We may be able to anticipate a future episode of rejection and initiate preemptive and personalized therapy and avoid damage to the kidney transplant altogether. That would be a great advance for both physicians and patients, and we are looking forward to conducting more research in this area to bring the test from the laboratory to the hospital.”

“We compared our results against the results of traditional biopsy tests and we found that our test could predict rejection of the kidney very reliably, with a level of accuracy around 80 percent at this early stage,” says Dr. Suhre, a professor of physiology and biophysics and the study’s lead author. “A great aspect of this research is that here in Qatar we have state-of-the-art equipment, high-tech computing power, and the expertise to carry out very detailed sample analysis, which complements the research of our colleagues in New York and allows us to conduct research together in a very synergistic way.”

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Managing Immunosuppression

In the years following the first successful kidney transplant in 1954, approaches to immunosuppression have evolved substantially, says David J. Cohen, MD, Medical Director of the Renal and Pancreatic Transplant Program. Dr. Cohen has long been involved in clinical protocols investigating new immunosuppressive drugs and combinations of existing medications for patients undergoing renal transplantation.

“The field is completely different than it was in the early years,” says Dr. Cohen. “Many would say that we are the victims of our own success in transplant. The current generation of drugs has enabled us to have excellent short-term success. However, long-term outcomes continue to be disappointing.”

While a regimen of immunosuppressant medications is vital for preventing the immune system from rejecting the donor organ, over time many of these drugs come with potentially serious side effects. “Some people get life-threatening infections, and some people will get cancer,” says Dr. Cohen.

According to Dr. Cohen, the field of transplant immunosuppression research has reached a crossroads. “Long-term studies are very difficult to do,” he says. “This has hampered the development of new transplant drugs, and the field has now evolved in some ways to an era focusing on diagnostics, looking to better understand the causes of allograft failure. Investigators in the field are also seeking to identify surrogate endpoints for long-term outcomes, such as predictive biomarkers, or the genetic make-up of the recipient so that a 10-year study may not be necessary.”

An epidemiologist and nephrologist in transplant medicine, Sumit Mohan, MD, MPH, Transplantation Nephrology, sees the goal of the Columbia program as two-fold. “One is that we want to improve the outcomes of patients who we transplant today with the tools that we have currently,” says Dr. Mohan. “At the same time, we are trying to improve access to kidney transplantation and thus improve outcomes for patients with end-stage renal disease.”

“Our approach to immunosuppression, at least initially, follows a relatively standard protocol,” says Heather K. Morris, MD, a nephrologist and transplant specialist who completed her fellowship in nephrology and her transplant nephrology training at Columbia. “We will start the majority of our patients on steroid-sparing protocols to minimize exposure to corticosteroids. Increasingly, we have been using belatacept, which can be given in place of calcineurin inhibitors for patients who do not tolerate tacrolimus well,” says Dr. Morris. “Tacrolimus is a wonderful drug that does a great job at preventing rejection. Unfortunately, it carries with it some adverse consequences. Many of our patients do quite well when their therapy is converted from tacrolimus to belatacept.”

Tacrolimus has been linked to an increased risk of developing diabetes following transplant, as well as the risk of malignancy. “Ironically, tacrolimus, perhaps our most effective immunosuppressive drug for kidney transplant recipients, can also cause nephrotoxicity,” says Dr. Morris. In particularly challenging cases, Dr. Morris and her colleagues occasionally will incorporate oncologic drugs, such as rituximab or bortezomib, for refractory antibody-mediated rejection.

Taking a Tolerant Approach

On the horizon, Dr. Cohen points to what he believes might be the Holy Grail in transplant advancement—tolerance. Columbia University researchers, in particular Dr. Morris under study leader Megan Sykes, MD, Director of the Columbia Center for Translational Immunology, have pinpointed the immune system mechanism that allows a kidney transplant to be accepted without lifelong immunosuppressive drugs, a significant step toward reducing or eliminating the need for costly and potentially toxic drugs and improving long-term transplant success. Their most recent findings were published in the January 2015 issue of Science Translational Medicine.

Using a new technique for identifying and tracking specific immune cells, combined with advanced genetic sequencing, the researchers found a set of patient-specific T cells that react to the donor tissue, increasing in number in patients who reject the organ but gradually disappearing in patients who accept the organ without immunosuppression and are therefore considered to be immunologically “tolerant” of their donors.

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**Education and Advocacy Efforts in Organ Donation and Allocation**

“Nearly 10 percent of the adult population in the United States has evidence of chronic kidney disease and most of them are asymptomatic and unaware that they have kidney disease,” says Dr. Mohan, whose efforts focus on lowering discard rates of kidneys and improving organ allocation.

According to Dr. Mohan, there is a huge demographic disparity in terms of who gets waitlisted for transplant. “We have shown that socioeconomically disadvantaged individuals and minorities, especially those who live in areas of intense poverty, are much less likely to be referred for transplantation, and, therefore, less likely to be waitlisted for transplantation. These individuals are also less likely to be able to identify a living donor,” continues Dr. Mohan.

“We know that African American recipients of kidney transplants do worse than non-African Americans,” says Dr. Mohan. “While some of the differences in outcomes have been attributed to socioeconomic barriers and factors such as patient education or compliance with complex medication regimens, recent studies have suggested that genetic risk factors such as the APOL1 mutation in donor kidneys is also a contributor.”

Dr. Mohan, with partners at other centers, recently published results that demonstrated the adverse impact of APOL1 donor genotype on long-term outcomes following transplantation. These findings provide additional evidence for determining the quality of a kidney prior to transplantation and improving the allocation of this precious resource.

“Dr. Mohan, along with collaborators, is also working to improve education efforts around kidney transplant. They recently completed a randomized controlled trial on the use of electronic shared decision making tools to help patients understand the survival benefits associated with transplantation. Additionally, they are developing patient and provider education material in a national NIH-funded study that includes over two-thirds of all dialysis patients in the United States to lower disparities in access to transplantation.

Improving an understanding of the quality of a kidney is also central to lowering the rate at which kidneys recovered from deceased donors end up not being transplanted and discarded. With nearly 17 percent of all recovered deceased donor kidneys being discarded, studies to improve understanding of organ quality and factors that contribute to the failure to use these kidneys are urgently needed. Dr. Mohan and his team recently published an analysis that highlighted the negative impact of weekends and the associated limited resources on transplantation rates in the United States.

“There are many complex ethical and public policy issues in the transplant arena,” says Dr. Cohen. “The most obvious public policy is support for basic research, but so is facilitating and removing barriers to live donor transplant, in particular financial barriers. Estimates are that the average live kidney donor incurs several thousand dollars of out-of-pocket expenses for such things as travel, lodging, and lost wages. The federal government has some programs that assist a small number of patients, but I think there needs to be a much bigger effort to recognize it. We need to make live kidney donation financially neutral for the donor.

“Kidney transplantation is an incredibly exciting field,” Dr. Cohen continues. “The basic science is fascinating, the ethical issues are challenging, and the clinical care is very rewarding. We are able to do wonderful things for people. We improve and prolong their lives.”

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**Reference Articles**


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