Pregnancy poses a serious challenge for maintaining normal maternal blood pressure. Pregnancy-induced hypertension, which occurs in about 10 percent of pregnancies, is a major cause of maternal and fetal deaths. During pregnancy, the uterus undergoes profound morphological changes, including trophoblast invasion and spiral artery remodeling. In preeclampsia, impaired spiral artery remodeling is common, but the underlying mechanisms are unclear.

Corin is a cardiac protease that activates atrial natriuretic peptide (ANP), a cardiac hormone important in regulating blood pressure. In mice, lack of corin prevents ANP generation and causes hypertension. In humans, corin variants are associated with hypertension. Unexpectedly, corin expression was detected in the pregnant uterus. As a transmembrane protein, corin is predicted to act at the expression sites, suggesting a possible function of corin in the pregnant uterus.

In a recent study published in Nature, we identified a novel function of corin and ANP in promoting trophoblast invasion and spiral artery remodeling. In this study, we created a mouse model in which a corin transgene was expressed under a cardiac promoter. The transgenic (Tg) and corin knockout (ko) mice were crossed to generate ko/Tg mice expressing corin only in the heart. In these mice, transgenic corin expression restored pro-ANP processing in the heart and normalized blood pressure, indicating that cardiac corin was sufficient to maintain normal blood pressure in nonpregnant mice. In pregnant corin ko/Tg mice that did not have pre-existing high blood pressure, blood pressure increased at approximately 17 days post-coitus and rose further before returning to the normal level after delivery. This phenotype resembled late gestational

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Welcome to the Nephrology Edition of Research Notes

The pursuit of groundbreaking scientific discoveries in the field of nephrology and hypertension at Cleveland Clinic’s Glickman Urological & Kidney Institute leverages critical collaborative research interaction, whether within our institute or linked with laboratories in the Lerner Research Institute.

In part because of the pathbreaking research that is conducted at Cleveland Clinic, both the urology and nephrology specialties were recognized by U.S. News and World Report as the top programs in the country.

In these pages, we highlight exciting research initiatives currently being conducted in the department that focus on understanding disease pathogenesis and identifying innovative treatment approaches in the areas of diabetic nephropathy, preeclampsia, polycystic kidney disease and kidney transplantation.

In our cover story, Qingyu Wu, MD, PhD, focuses on the role of the cardiac hormone corin and atrial natriuretic peptide in promoting trophoblast invasion and spiral artery remodeling to prevent hypertension in pregnancy. The laboratory of Oliver Wessely, PhD, is actively pursuing the transformation of normal renal epithelial cells into precystic cells utilizing both mouse and African clawed frog models. Amin Wang, PhD, and collaborators have uncovered a unique regulatory mechanism for mesangial expansion and glomerular macrophage infiltration in diabetic nephropathy that will potentially lead to future therapeutic interventions. Robert Fairchild, PhD, and Emilio Poggio, MD, provide insight into critical mechanisms of rejection in renal allografts. And the clinical team in transplant, dialysis and chronic kidney disease are rethinking our practice in several key areas.

It has been my distinct honor to serve as Chairman of Nephrology and Hypertension for the past seven years. I am immensely proud of what we have accomplished, both clinically and scientifically, during my tenure. After much consideration, I believe that the time is right to transition to a future leader who will continue advancing the legacy of the department. I am committed to working with Cleveland Clinic as we identify the very best candidate to lead the department forward and as we welcome Robert Heyka, MD, who assumes the role of Vice Chair of the institute and Department Chair on an interim basis.

I hope you find this research inspiring to you in your practice.

Sincerely,

Martin Schreiber, MD
Vice Chairman
Glickman Urological & Kidney Institute
Department Chairman
Nephrology and Hypertension
schreim@ccf.org
In pregnancy, trophoblast invasion and uterine spiral artery remodeling are important for lowering maternal vascular resistance and increasing uteroplacental blood flow.

Here we identify a novel function of corin and ANP in promoting trophoblast invasion and spiral artery remodeling. These results indicate that corin and ANP are essential for physiological changes at the maternal-fetal interface, suggesting that defects in corin and ANP function may contribute to preeclampsia.

Proteinuria is a hallmark of preeclampsia. Wild type, corin ko and ko/Tg mice had similar urinary protein levels before pregnancy and at midgestation. The levels, however, increased in corin ko and ko/Tg mice at late gestation, consistent with reported proteinuria in mouse models of preeclampsia. Ischemic glomeruli, indicated by fewer red blood cells, were found in pregnant corin ko and ko/Tg mice, but not in nonpregnant mice. PAS staining revealed increased extracellular matrices and collapsed glomerular capillaries in pregnant corin ko and ko/Tg mice. Electron microscopy showed narrow glomerular capillary lumens and thick basement membranes, suggesting endotheliosis and increased extracellular matrices. Additional pathological features such as necrotic cells and calcium deposits in the placental labyrinth also existed in these mice, indicating insufficient uteroplacental perfusion. Consistently, corin ko and ko/Tg mice had smaller litters. We also showed that trophoblast invasion and spiral artery remodeling were impaired in corin ko and ko/Tg mice and that this defect occurred before blood pressure increased in these mice.

Pro-ANP is expressed in the uterus. If corin acts on pro-ANP to promote trophoblast invasion and spiral artery remodeling, thereby preventing hypertension in pregnancy, ANP and corin ko mice should have similar phenotypes. Indeed, we found similarly increased blood pressure in pregnant ANP ko mice. The mice also had late gestational proteinuria and smaller litters. Thus, ANP and corin ko mice had very similar phenotypes, indicating that the role of corin in pregnancy is likely mediated by ANP.

ANP is known to relax vascular smooth muscles. Recently, ANP and its downstream cGMP-dependent protein kinase were shown to be important in angiogenic processes by promoting endothelial regeneration. Thus, ANP may function locally to remodel uterine arteries and promote trophoblast invasion. In a cell-based experiment, we found that ANP markedly stimulated human trophoblasts to invade Matrigel™ matrices. In these cells, ANP receptor mRNA expression was confirmed and ANP-stimulated intracellular cGMP production was detected.

Our findings underscore the importance of ANP produced locally by corin, which acts on trophoblasts and vascular cells in the uterus. Because heart-derived ANP circulates inside the vessel, our model may explain why cardiac corin failed to promote trophoblast invasion and uterine artery remodeling, as shown in corin ko/Tg mice. To verify this hypothesis, we quantified corin mRNA and protein in human uteruses by RT-PCR and ELISA. The levels were low in nonpregnant women but increased in pregnant women. In preeclamptic women, the levels were significantly lower than in normal pregnancies. These results support a local corin function in the pregnant uterus.

We then sequenced the corin gene in preeclamptic patients and identified a mutation that altered Lys to Glu at position 317 in LDL receptor repeat 2 in one woman and another mutation that altered Ser to Gly at position 472 in frizzled 2 domain in two women from the same family who had preeclampsia. The data were consistent with previous findings that LDL receptor repeats and frizzled domains are critical for corin activity, suggesting that the mutations may impair corin function in the patients, thereby contributing to preeclampsia. Interestingly, corin variants in frizzled 2 domain that impaired corin function have been reported in blacks, a high-risk population for preeclampsia.

In summary, we have identified a novel local function of corin and ANP in promoting trophoblast invasion and spiral artery remodeling to prevent hypertension in pregnancy. Our data suggest that impaired corin expression or function in the pregnant uterus may represent an important mechanism underlying preeclampsia.

Dr. Wu is staff in the Lerner Research Institute. He can be reached at wuq@ccf.org or 216.444.4351.

Polycystic kidney disease (PKD) is among the most abundant single-gene disorders in humans. Its most frequent subtype, autosomal dominant PKD, affects about 1 in 800 people in the U.S. It is characterized by the formation of fluid-filled cysts that accumulate and expand over time, thus causing kidney malfunction and eventually leading to kidney failure. Contrary to its name, PKD not only affects the kidneys but is actually a multiorgan disease that also leads to the development of liver cysts, cardiovascular complications and intracranial aneurysms, among other comorbidities.

Importantly, even though the genes mutated in human patients, polycystin-1 and polycystin-2, have been cloned for several years, the underlying molecular mechanisms of cyst development are still poorly understood. As a consequence, dialysis and kidney transplantation are still the only approved therapeutic options.

Most current approaches to develop new drugs to treat PKD focus on preventing the expansion of kidney cysts by inhibiting either inhibiting cell growth or fluid accumulation in the cysts. While such approaches are clinically relevant and can postpone or even alleviate the necessity to clinically interfere with the disease, it only treats the symptoms and will never cure the entire spectrum of PKD symptoms.

My laboratory is tackling this problem by focusing on the initial events that trigger the transformation of a normal renal epithelial cell into a precystic one. We believe that concentrating on the first steps leading to cyst formation will result in the discovery of new angles for treating PKD in the future. The hope is that this focus will provide novel therapeutic approaches.

To this end, my laboratory is using a two-model system approach, combining a mouse model with the African clawed frog, *Xenopus laevis*. While the metanephric kidney in the mouse has been the model system of choice to study PKD, my group was the first to establish the amphibian pronephros as an alternative. With its fast development (a functional kidney forms within two days), its ease of molecular manipulations and the large numbers of eggs laid in one session, the Xenopus is an ideal companion system to the study of PKD in mice, where genetic studies can easily take a year before definitive answers are obtained. Using the distinct advantages of both organisms greatly facilitated the formulation and experimental testing of a new hypothesis about the role of Bicaudal-C in the kidney and its contribution to the formation of PKD.

### Bicaudal-C as a Post-transcriptional Regulator

Among the several projects in my laboratory that study these early steps of cystogenesis in PKD, the one studying Bicaudal-C (BicC1) best illustrates the potential of the approach and the possibilities for novel therapeutic areas. BicC1 encodes an RNA-binding molecule, first identified in Drosophila as a protein regulating anterior-posterior development. We identified the Xenopus and mouse homologues of BicC1. Interestingly, mouse mutants of BicC1 and Xenopus embryos lacking BicC1 develop kidney abnormalities reminiscent of PKD. However, it was unknown why BicC1 is required to prevent renal cyst formation and how it relates to other molecules that are involved in PKD.

To address the molecular mechanism of BicC1 activity, my laboratory performed an in-depth analysis in BicC1 mutant mice. Knockout of BicC1 in mice resulted in the formation of cysts along the entire length of the nephron, a mouse phenotype remarkably similar to the one of polycystin-1 or polycystin-2 mouse mutants. These epithelial malformations were not caused by increased proliferation.

Instead, using the pronephric kidney of Xenopus and the metanephric kidney of mouse, we could show that BicC1 regulated the expression of polycystin-2, one of the proteins causing PKD in humans. Molecular analyses demonstrated that this effect of BicC1 was caused in a completely unexpected way. BicC1 protein was localized to cytoplasmic foci that were positive...
for P-body markers such as GW182. These foci have previously been shown to be the sites of post-transcriptional regulation by a novel class of molecules, small noncoding RNAs termed microRNAs (miRNAs). These miRNAs bind to the 3’ UTR of a given gene and regulate mRNA stability and/or translation.

Indeed, studies in my laboratory have shown that Polycystin-2 mRNA is regulated by the miR-17 miRNA family and that this repressive activity is antagonized by BicC1. Most important, the in vivo relevance of this mode of action was confirmed, again using the Xenopus PKD model. The pronephric kidney defects caused by loss of BicC1 were rescued by reducing miR-17 activity. Based on these data, I proposed that the PKD phenotype in BicC1 mutant mice is caused by dysregulation of a miRNA-based translational control mechanism. This work demonstrated - for the first time - that post-transcriptional regulation is important in maintaining epithelial structures and that the disruption thereof can result in PKD formation.

One intriguing aspect of these findings is that miRNAs have emerged as a novel class of cellular regulators that frequently function as rheostats, adjusting cells to different environmental conditions. As such, their expression is often altered under disease conditions. This has resulted in a growing field that uses miRNA as biomarkers for disease progression. Furthermore, correcting those expression levels has emerged as a novel therapeutic option, particularly in cancer therapy.

In the case of PKD, similar approaches are still in their infancy. My laboratory is currently gearing up to test the feasibility of the approach using the BicC1 mutant mice for a proof-of-principle experiment.

Dr. Wessely is a staff member in the Department of Cell Biology and the Glickman Urological & Kidney Institute. He can be reached at wesselo@ccf.org or 216.444.3050.

For references, please email the author.
A Novel Model to Study Mechanisms Underlying Antibody-Mediated Rejection of Renal Allografts

By Robert Fairchild, PhD

The use of current immunosuppressive strategies has markedly decreased the incidence of T cell-mediated acute rejection in transplant patients. In contrast, the detected incidence of antibody-mediated graft rejection in solid organ recipients is increasing.

Acute humoral rejection (AHR) occurs in approximately seven percent of renal transplant patients and is frequently observed in cardiac and lung graft recipients. Donor-specific antibodies can directly mediate injury to graft endothelium as well as promote the development of graft tissue fibrosis and vasculopathy. Recent recognition of the high incidence of AHR has generated considerable interest in defining mechanisms by which donor-reactive anti-class I MHC and anti-class II MHC antibodies mediate graft tissue injury. A major problem that has confronted investigation of these mechanisms is the absence of appropriate animal transplant models.

As with human transplant patients, the alloimmune response to skin and organ allografts in nonimmunosuppressed recipients is primarily mediated by T cells with little or no contribution by donor-reactive antibody. Prior sensitization of organ allograft recipients by transplanting donor skin grafts prior to the organ transplant results in the generation of memory CD4 and CD8 T cells as well as donor-reactive antibody, and it is difficult to discern the specific effects of the antibody in the rejection of the organ allograft. As an alternative approach to the use of presensitized recipients, donor-reactive polyclonal or monoclonal antibodies can be infused in organ allograft recipients. However, drawbacks to this approach include the sudden administration of a bolus of the antibody and any limitations on the time period during which the antibody is given to the recipient.

We recently observed marked increases in serum levels of donor-reactive antibody induced in complete MHC-disparate heart and kidney allografts in murine recipients lacking expression of the chemokine receptor CCR5. These dysregulated antibody responses in CCR5-/- recipients appear more quickly and have titers 15-50-fold higher than those observed in wild-type C57BL/6 recipients. The consequence of these antibody responses is acute humoral rejection of the grafts that is accompanied by intense C4d/C3d deposition in the capillaries and large vessels of the allograft (Figure 1). The histopathology of the response looks identical to that observed during antibody-mediated rejection of renal grafts in human transplant patients.

An advantage of this model is that the antibody-induced pathology in the renal allograft can be followed from the beginning of the donor-reactive antibody response, that is first detectable in the recipient serum about five days after the transplant, and thereafter when the donor-reactive antibody titers rapidly increase. In addition, we have now established a colony of CCR5-/- mice that is further genetically manipulated so that all B cells express the human CD20 protein, allowing depletion of the B cells at any time before or after the transplant by administration of the anti-human CD20 antibody currently used to treat antibody-mediated rejection in clinical transplantation.

Studies of serum from heart and renal transplant patients experiencing AHR indicate that antibodies to both donor class I and class II HLA antigens can cause graft injury, but that injury mediated by class II MHC-specific antibodies is characterized by more intense infiltration of neutrophils and other inflammatory components in the allograft.

We have recently used the CCR5-/- mouse model to investigate the pathology induced by antibodies reactive to donor class I MHC molecules by testing the rejection of heart allografts transgenically expressing a single class I MHC disparity in the CCR5-/- recipients. Whereas the single class I MHC disparate allografts survived longer than 60 days in wild-type recipients, all CCR5-/- recipients rejected the allografts within 14 days, and this rejection was mediated by antibody reactive to the donor class I MHC disparity.

Key Points:
Recent recognition of the high incidence of AHR has generated considerable interest in defining mechanisms by which donor-reactive anti-class I MHC and anti-class II MHC antibodies mediate graft tissue injury.

Administration of the anti-human CD20 antibody to renal allograft recipients at the time the donor-reactive antibody first becomes detectable in the serum (e.g., Day 5 post-transplantation) results in the long-term survival of the renal allografts, but beginning about Day 50, the grafts begin to develop interstitial fibrosis and vasculopathy that further increase with time.
During the course of these studies, we observed that the antibody-mediated rejection of the single class I MHC disparate grafts was accompanied by the high-level expression of three novel markers in the graft: perforin, myeloperoxidase and CCL5 mRNA. Since this rejection occurs in the absence of T cell-mediated rejection mechanisms, these studies indicate that the donor-reactive antibody directly induces the expression of these three biomarkers.

These studies raise questions about the antibody-mediated mechanisms inducing the expression of these molecules that have effector functions in innate and adaptive immune responses. We now approach these questions by using immunohistochemical staining of rejecting grafts to identify the sources of the perforin, myeloperoxidase and CCL5. In addition, we are generating CCR5-/- mice that cannot express these molecules in order to test their role in antibody-mediated rejection of heart and kidney allografts. Finally, we have developed an approach to detect the presence of these molecules in the urine of renal allograft recipients during antibody-mediated graft rejection and are applying this non-invasive detection strategy to human kidney transplant patients.

A major problem in solid organ transplantation continues to be the development of interstitial graft fibrosis and occlusive vasculopathy that occurs as a consequence of chronic injury imposed on the graft. Either donor-reactive T cell or antibody responses or both can initiate this pathology but mechanisms underlying the initiation and progression of this pathology are poorly understood. We have used our CCR5-/- mice expressing the human CD20 protein on B cells to investigate this problem. Administration of the anti-human CD20 antibody to renal allograft recipients when the donor-reactive antibody first becomes detectable in the serum (e.g., Day 5 post-transplantation) results in the long-term survival of the renal allografts, but beginning about Day 50 the grafts begin to develop interstitial fibrosis and vasculopathy that further increase with time. Using this approach, we have begun to dissect the molecular and cellular mechanisms underlying the donor-reactive antibody induction of this pathology.

We have developed a novel model that allows more precise analysis of the mechanisms and consequences of donor-reactive antibody mediated rejection of renal allografts. This model is being used to discern differences in the pathologies induced by antibodies specific for various donor MHC molecules, in the development of pathology resulting from chronic injury, and in the development of novel approaches to detect and monitor acute and chronic graft pathologies resulting from such antibodies.

Dr. Fairchild is Director of the Transplant Immunology Laboratory. He can be reached at fairchr@ccf.org or 216.444.3146.

For references, please email the author.
Kidney transplantation is the treatment of choice for patients suffering from end-stage renal disease because it provides better quantity and quality of life than other means of renal replacement therapy. Despite tremendous advances in the medical and surgical management of these patients, the short- and long-term success of this procedure is still not ideal, and therefore the research and clinical transplant communities are engaged in a constant effort to further understand and learn the underlying biological mechanisms that preclude the desired outcomes. Advances in the scientific field are constant but slow, and there is an increasing interest in studying new noninvasive biomarkers that will help the clinician predict or prevent poor outcomes, providing new opportunities for early interventions or development of new therapeutic strategies.

Novel biomarkers and techniques are rapidly emerging in the field of basic kidney transplantation research; however, translation into human research is lacking. Some of these biomarkers have been studied and are close to routine clinical implementation, but the vast majority are at different stages of development and are not yet available.

Furthermore, these new biomarkers are still to be measured and validated in large cohorts of kidney transplant recipients. The advent of new techniques to study the pathogenesis of immune-mediated injury, such as organ transplant rejection, will require repetitive tissue sampling in a large cohort of patients to assess their potential clinical application to patient care.

A large number of human samples and state-of-the-art laboratories are required to test and validate these biomarkers and to study mechanisms of disease. Thus, there is a need to systematically collect samples and store those samples in biobanks/biorepositories for future investigations.

Transplant Program has established this needed biorepository, which we have named the Renal Transplant BioBank.

The goal of the Renal Transplant BioBank is to establish a patient-linked biologic sample repository (blood, urine and graft histology) for use in future research studies related to metabolic, inflammatory and immunologic markers relevant to organ transplantation. However, the first step is to build up the repository, which is labor-intensive and costly. In collaboration with the Lerner Research Institute, the Kidney and Pancreas Transplant Program, with financial support provided by the Novick Center for and Translational Research in the Glickman Urological & Kidney Institute, has initiated the collection and storage of biospecimens (blood, urine, biopsy tissue) from kidney and pancreas transplant recipients who receive an organ at Cleveland Clinic. The collection is initiated after the patient has provided informed consent.

These biospecimens are intended to be used in the future to develop and test novel biomarkers that will eventually translate into better patient care. This is one of the most relevant endeavors in the research arena of the program due to its potential future application. Importantly, this is a unique initiative that is possible only in a few medical centers in the U.S. Finally, the experience gained in this endeavor will be the basis for future expansion of this type of biorepository to other areas in kidney disease.

Dr. Poggio is a staff member in the Nephrology and Transplantation sections of the Glickman Urological & Kidney Institute. He can be reached at poggioe@ccf.org or 216.444.5383.

Key Points:
Kidney transplantation is the treatment of choice for patients suffering end-stage renal disease, yet the short- and long-term success of this procedure is still not ideal.

The research and clinical transplant communities are engaged in a constant effort to further understand and learn the underlying biological mechanisms that preclude the desired outcomes.

A large number of human samples and state-of-the-art laboratories are needed to test and validate these biomarkers and to study mechanisms of disease, and thus there is a need to systematically collect samples and store those samples in biobanks/biorepositories for future investigations.
Diabetic nephropathy (DN) is the leading cause of end-stage renal disease. In general, DN can be divided into two stages: 1) the genesis stage with early cellular, structural and functional changes; and 2) the progression stage with advanced structural and functional changes leading to irreversible renal failure. Mesangial expansion is the principal glomerular lesion in DN that reduces the area for filtration and leads eventually to sclerosis and renal failure.

However, the mesangial extracellular matrix expansion and sclerosis are preceded by an early phenotypic activation and proliferation of the glomerular mesangial cells, followed by a prominent glomerular infiltration of monocytes and macrophages associated with glomerular hyaluronan matrix formation. Glomerular monocytes and macrophages have been prominently identified in DN in both animal models and humans, and appear to have a key role in the induction of mesangial matrix expansion by elevating glomerular TGF-beta, hypercellularity, and the onset of proteinuria, which are characterized by inflammatory processes that are not yet clearly understood. Our studies focus on the hyaluronan-based matrix that is synthesized by dividing cells stressed by hyperglycemia, and on its dialogue with associated macrophages, which are derived from circulating monocytes that are recruited into the inflamed tissues. We believe that this monocyte-adhesive hyaluronan matrix and its interactions with the macrophages have a central role in defining the resulting inflammatory pathologies, and that understanding the mechanisms involved will identify ways to ameliorate or prevent the pathological consequences.

Further, under hyperglycemia, elevated glucose metabolites (UDP-sugars) are major contributors to pathological responses. Our studies will also reveal significant new insights regarding the potential therapeutic roles of the heparin trisaccharide or oligosaccharides by decreasing intracellular UDP-sugars based on the formation of the hyaluronan matrix and the glomerular macrophage infiltration in DN.

Key Points:
1. Glomeruli isolated from kidneys of rats over a six-week period after initiating the diabetic response showed increased numbers of mesangial cells that underwent autophagy, a continuous increase in hyaluronan content and an influx of macrophages, with resulting nephropathy and proteinuria by six weeks.
2. Our new data show that the presence of 0.2 microM heparin in hyperglycemic medium prevented the intracellular hyaluronan synthesis and the autophagic response in dividing mesangial cells.
3. Our most recent data show that a heparin trisaccharide with a nonreducing terminal O- and N-sulfated glucosamine initiates the same reactions as heparin in mesangial cells stimulated to divide in hyperglycemic medium.

Figure 1. Model for synthesis of hyaluronan by hyaluronan synthases in normal cell biology. The confocal images are of live COS-1 cells transfected with GFP-Has3. The green shows where the enzyme is in intracellular compartments. The yellow shows where the enzyme is in the plasma membrane and actively synthesizing hyaluronan (red).
Hyaluronan, a high molecular weight (MW) polysaccharide, is normally synthesized at the plasma membrane by hyaluronan synthases that use cytosolic UDP-sugar substrates and extrude the growing polymer into the extracellular space (Figure 1). Thus, it is important for the synthases to be embedded in the plasma membrane before they are activated.

Our studies showed that rat mesangial cells that divide in hyperglycemic (25.6 mM) glucose activate hyaluronan synthases inside the cell before reaching the plasma membrane. This induces synthesis in intracellular compartments – endoplasmic reticulum (ER), transport vesicles, golgi (Figure 2). The deposition of the high MW, polyanionic hyaluronan polymer in the ER initiates a novel ER stress autophagy that upregulates cyclin D3 at the end of cell division, and that cyclin D3 activates an autophagosome process that extrudes hyaluronan to form a monocyte-adhesive extracellular matrix.

This process occurs in glomeruli of streptazotocin diabetic rats. Glomeruli isolated from kidneys of rats over a six-week period after initiating the diabetic response showed increased numbers of mesangial cells that underwent autophagy, a continuous increase in hyaluronan content and influx of macrophages with resulting nephropathy and proteinurea by six weeks (Figure 3).

This pathological hyaluronan response of dividing cells to hyperglycemic medium has been demonstrated in aortic smooth muscle cells and in 3T3-L1 cells, which have been used extensively as a model for normal adipogenesis. It is likely to be centrally involved in many diabetic pathologies, where adipogenesis and pathological matrices are common.

Studies by Gambaro et al. showed that a daily IP injection of a small amount of a low-MW heparin preparation in the diabetic rat model prevented the nephropathy and proteinurea over an eight-week period. Our new data show that the presence of 0.2 microM heparin in hyperglycemic medium prevented the intracellular hyaluronan synthesis and the autophagic response in dividing mesangial cells. However, at the end of cell division, the cells produced approximately threefold more monocyte-adhesive extracellular hyaluronan matrix than cells dividing in hyperglycemic medium alone. This process occurs in diabetic rats that were treated daily with the heparin (Figure 3). The hyaluronan content of the glomeruli from the treated rats increased greatly during Weeks 1-2 and then decreased to near normal levels by Week 6. The animals did not have the nephropathy or proteinurea. The glomeruli in sections from the Week 6 kidney showed no autophagy and very little hyaluronan but contained a large number of macrophages.

Our most recent data show that a heparin trisaccharide with a non-reducing terminal O- and N-sulfated glucosamine initiates the same reactions as heparin in mesangial cells stimulated to divide in hyperglycemic medium. This trisaccharide, unlike heparin, does not have anticoagulant activity and will be a valuable reagent to study the mechanisms involved in these processes in both in vitro and in vivo models.
Future Perspective

Our hypothesis is that dividing cells have a cell surface receptor that interacts with this heparin trisaccharide whereas nondividing cells do not. This is supported by our previous studies that showed that heparan sulfate oligosaccharides generated by heparanase cleavage during catabolism of heparan sulfate proteoglycans bound to mesangial cells showed arrested growth at the G0/G1 cell division stage, but did not do so in confluent mesangial cells. Heparin also bound similarly and showed a Kd of 1.6 x 10^-8 M. The mammalian heparanase is an endoglucuronidase that exposed nonreducing terminal sulfated glucosamines, consistent with our results with the heparin trisaccharide.

Our future studies will focus on 1) identifying this receptor; 2) determining the signaling pathway that activates the hyaluronan synthases inside the dividing cell that is blocked in the presence of the heparin trisaccharide; and 3) determining the signaling pathway that greatly activates hyaluronan synthase activity in the plasma membrane in the presence of the heparin trisaccharide, which forms the extensive pathological monocyte-adhesive hyaluronan extracellular matrix.

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For references, please email the author.
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