Klinik für Nieren- und Hochdruckerkrankungen

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Forschungsprofil

Die wissenschaftlichen Schwerpunkte der Klinik für Nieren- und Hochdruckkrankheiten liegen auf verschiedenen Gebieten des Faches: in der Nephrologie sind es die Krankheiten diabetische Nephropathie, die Vaskulitiden mit Nierenbeteiligung sowie die Probleme der transplantierten Niere. Therapeutisch werden neue Verfahren in der Dialyse sowie der Immunadsorption untersucht. Auf dem Gebiet des Bluthochdrucks sind es die renovaskuläre Hypertonie und die therapie-resistenten Hypertonieformen. Hier werden neue Verfahren der Bildgebung sowie therapeutisch invasive Strategien wie Sympathikusablation und Karotisstimulation erforscht. Die Abteilung ist erfolgreich in der Planung und Durchführung prospektiver Studien auf dem Gebiet der Nierentransplantation, der diabetischen Nephropathie und der Hypertonie. Pathophysiologisch stehen die Untersuchungen zu den molekularen Mechanismen der Proteinurie sowie die Mechanismen der akuten und chronischen Gefäßschädigung im Vordergrund. Die Funktion der Podozyten einerseits sowie der Endothelzellen andererseits werden in verschiedenen Tiermodellen (Maus, Zebrafisch) analysiert. Das Endothel und seine Interaktionen mit Leukozyten spielt auch bei Untersuchungen zum Ischämie/Reperfusions-Schaden, zur Sepsis und bei der chronischen Transplantatdysfunktion eine wichtige Rolle. Weitere Schwerpunkte der experimentellen Forschung in der Abteilung sind die Mechanismen der interstitiellen Fibrose, der Wirkung von Proteasen sowie der Entstehung chronischer Gefäßschäden. Für die erfolgreiche Forschung in unserer Klinik sind die Kooperationen innerhalb der MHH von großer Bedeutung. Hervorzuheben sind die Zusammenarbeit innerhalb des IFB-Tx mit den Core Facilities (Falk, Koehl, Thum) und dem Clinical Research Center. Es bestehen weiterhin enge Kooperationen mit den Abteilungen Klinische Pharmakologie, der Radiologie, der Klinik für Kinderheilkunde, der Klinik für Kardiologie und dem Institut für Pathologie.

Forschungsprojekte

"Zebrafishing" for Novel Genes Relevant to the Glomerular Filtration Barrier

Chronic kidney disease (CKD) is a national and world healthcare priority. CKD is rarely detected early enough in patients, typically leads to kidney failure, and frequently requires therapy through dialysis or transplantation. Proteinuria is one of the clinical hallmarks when diagnosing CKD. The need for therapies is considerable; presently, we are only able to treat the sequel of CKD, hypertension, and metabolic disease. In order to discover novel therapeutic strategies, we have to understand the molecular mechanisms of the disease and identify novel targets.

The generation of a murine model used to identify mech- anisms and novel genes relevant to CKD and proteinuria is time consuming, is very costly, yields only a small number of test subjects, and can have other serious shortcomings such as embryonic mortality and failure to generate phenotypes, or generate nonkidney specific phenotypes. However, zebrafish are an ideal model to screen novel genes relevant to glomeru- lar filtration barrier function or proteinuria. Using zebrafish, we are able to screen entirely novel genes in 4-6 weeks in hundreds of live test subjects at a fraction of the cost of a mammalian model. Zebrafish develop from a fertilized egg to free-swimming larvae in only 48 hours and develop a fully functional kidney unit within 72 hours, and effects can be monitored within 2-3 days after fertilization. Protein production in zebrafish larvae can easily be influenced by specific gene knockdown or overexpression

techniques with a morpholino approach. However, proof of specific and definite alteration of protein expression level has to be given for each experiment. This paper covers these basic techniques and describes the zebrafish model as a simple and fast screening system to identify genes relevant for the integrity of the glomerular filtration barrier. Genes of interest are „knocked down" by morpholino injections and are functionally characterized by (1) edema formation and (2) urinary albumin excretion. Using our approach the question of functional relevancefor a given gene of interest can be answered within a time frame of 4-6 weeks, and this evidence can be the basis for further analysis in rodent models or human tissues.

Analysis of Renal Phenotypes: The Specificity of Generalized Edema

A first hint of a renal phenotype is the development of generalized edema in the zebrafish larvae. After injecting a morpholino or mRNA into the 1-2 cell stage zebrafish embryos, larval development is monitored for 120 hours postfertilization (hpf), and phenotype development and mortality are recorded. For uninjected wildtype and control (scrambled oligonucleotide) injected embryos, the percentage of fish that develop severe generalized edema should be significantly lower. If a high percentage (>40%) of embryos develop edema in the knockdown or overexpression group within the first 120 hpf, this could be a first indication of a renal phenotype. In contrast, nonspecific edema can be observed in small percentages (<3-5%) of uninjected genetically unmodified embryos.

We classify zebrafish embryo edema phenotypes for qualitative and quantitative analysis in a range from phenotype I (PI) to phenotype IV (PIV) (Figure 1). To ensure that the quality of the eggs did not influence the phenotype develop- ment, it is always important to have a significant number of uninjected wildtype fish from the same clutch that were used for a specific injection. Uninjected and control morpholino - injected fish should develop a healthy looking slim shape (PI phenotype). In contrast, if the genetic modification leads to an edema as part of the phenotype, a higher percentage of knockdown morpholino-injected fish will develop severe generalized edema with pericardial effusion and yolk sac edema (PII to PIV).

The edema in zebrafish may range from mild edema (PII phenotype) to severe (PIII) or extremely severe (PIV). However, edema is considered to be nonspecific, since a small percentage of wildtype fish develops mild edema as well as a sign of developmental defects, mostly in the cardiovascular system. If a significant portion of genetically modified fish develop edema, the phenotype can only be interpreted as a first hint that the kidney is affected due to multiple causes of fluid accumulation in one or more cavities of the body. Generalized edema can be due to a rise in hydrostatic pressure caused by cardiac failure, a decrease in plasma oncotic pressure within blood vessels in nephrotic syndrome, or liver failure. Proteinuria is responsible for the development of hypoproteinemia and decreased plasma oncotic pressure. Therefore, plasma water translocates from the intravascular space into the interstitial tissues. Thus, it is important to confirm that the edema detected in zebrafish embryos is related to kidney disease. To differentiate between cardiac and renal phenotype, we established the following screening assays for zebrafish.

Proteinuria Screening in Zebrafish

To establish that the observed edema phenotype is associated with the loss of high-molecular-weight proteins, we established novel assay systems: the tubular protein detection assay, the FITC labeled dextran assay (FITC-eye- assay), and the Tg(l-fabp:DBP-eGFP) assay (Fabp-eye-assay).

The zebrafish embryonic pronephros consists of two nephrons with glomeruli fused at the embryo midline and two pronephric tubules that connect the glomerulus to the pronephric ducts, that fuse just before the cloaca [1]. As in the mammalian kidney, the function of the pronephric tubules is to reabsorb essential proteins that are small enough to have passed through the glomerular filtration barrier. If the glomerular filtration barrier is compromised, normal or low-molecular-weight proteins as well as larger than 70 kD proteins can also pass through the glomerular filtration barrier and are then reabsorbed in the tubules. To document the reabsorption of high molecular weight proteins, the first assay we use is a tubular protein detection assay. We use the transgenic wt1b:EGFP zebrafish line that exhibits

two orthologs found in teleost species such as human Wilms tumor gene 1 (WT1), wt1a, and wt1b [2]. GFP expression in the wt1b:eGFP line can be observed in the pronephros starting at 17 hpf, at 35 hpf expression is detected in the pronephric glomeruli, tubules, and part of the ducts, and at 50 hpf the pronephros is fully functional and filtering blood. At this time the wt1b expression has migrated to the midline but has not fused, indicating that the expressing cells are found on the tubular pole of the glomerulus at the neck region of the proximal tubule [3]. GFP labeled Wt1b in the tubules of a 72-hour-old zebrafish is an excellent model to observe reabsorption of proteins in the tubules.

Tubular Protein Detection Assay. To perform the tubular assay Wt1b:EGFP, zebrafish are mated, and the gene of interest morpholinos and the control solution are injected as described previously. The chorions are removed from the embryos manually with forceps at 48 hpf, and the embryos are ready for cardinal vein injection at 72 hpf. The anesthetized fish are positioned dorsally in a v-shaped agarose injection- mold. A 70 kDa rhodamine-labeled dextran is injected into the cardiac venous sinus. After injection, the embryos are moved into fresh embryo raising medium (ERM) and allowed to recover from anaesthesia. Embryos are placed in the 28.5°C incubator until imaging at 120 hpf. For imaging, the embryos are reanesthetized and imaging can be performed in live animals with a confocal microscope. If the glomerular filter has a barrier defect, significant amounts of red-fluorescent 70 kD rhodamine-dextran will be detectable inside the green fluorescent proximal tubular cells. This assay has several advantages: the fish are still alive and developing so imaging can be performed before or after the 120hpf time point, tubules can be examined for glomerular leakage; normal development and the fusing zebrafish pronephros can be documented. Documenting the fusion of the pronephros is an important control, since severe developmental defects can be the sign of a developmental phenotype that affects normal kidney development.

Fluorescence Eye Assays. For differentiation between cardiac renal origin of the observed edema and we established eye assay models for indirect measuring of the integrity of the glomerular filtration barrier. Both systems share the concept that under normal conditions high-molecular-weight plasma proteins are retained in the circulation of the fish. If these plasma proteins are fluorescence labeled, they can be monitored over the retinal vessel plexus as representative locations for systemic fluorescence. A decreasing fluorescence level in the eye (e.g., after morpholino gene knockdown) supports our hypothesis of leakiness of the glomerular filtration barrier with loss of (fluorescence labeled) high-molecular-weight protein into the water. We use two different eye assay systems to detect if high-molecular-weight molecules pass through the glomerular filtration barrier: the FITC-eye-assay and FABP-eye-assay.

For the FITC assay, AB zebrafish can be mated, and the collected embryos are injected with both a targeted morpholino and a control morpholino at the 1-2 cell stage as described above. Dechorionated embryos at 48hpf are then anesthetized and prepared for cardinal vein injection as described above. In this assay, we inject the fish with 4.6 nL of 70 kD FITC labeled dextran, allow them to recover and transfer them individually with 200 µL of ERM into a 96 well plate, and maintain in a 28.5°C incubator. At 24 and 48 hours postinjection (hpi), images of the retinal vessel plexus of anesthetized fish are captured using an inverted microscope. The maximum fluorescence intensity is analyzed using the NIH ImageJ program in the fish retinal pupil [46]. We observe that fluorescence levels from 24 to 48 hpi uninjected wild type and control morpholino-injected fish remain steady or even increase. Increased intensity is likely to be due to continual uptake of the 70 kDa dextran [4]. However, if a genetically modified fish demonstrates a significant decrease in fluorescence intensity at 24-48hpi, this indicates that the glomerular filtration barrier is compromised and is allowing the 70 kDa proteins to pass through (Figure 2). This technique can be used for morpholino injected as well as for any mutant fish line that has to be tested.

Tg(l-fabp:DBP:EGFP) fish, which were initially generated to visualize the blood brain barrier in vivo are used in the Fabp-eye-assay [5]. These fish express a vitamin D binding protein fused with the enhanced green fluorescent protein (DBP-EGFP) under the control of the liver-type fatty acid binding protein (l-fabp) promoter [5]. The DBP-eGFP fusion protein has a molecular weight of approximately 78 kDa. Serial images of the retinal vessel plexus show a steady

increase of fluorescence levels at 96 hpf, 120 hpf, and 144 hpf in a nonmorpholino-injected Fabp Tg fish. Similar to the assays above, the fish are mated and the eggs are injected at the 1-2 cell stage with a targeted morpholino or control morpholino and allowed to grow. A second injection step is not necessary in this fish line, making them a convenient model system to study effects in knockdown or overexpression systems. Following the imaging protocol used in the FITC assay, images of the eye are taken at 96, 120, and 144 hpf and analyzed using Image J. In contrast to the increasing fluorescent levels seen in the uninjected wild types and control morpholino-injected fish, the experimental morpholino-injected fish show a lower overall fluorescence that barely increases over time (Figure 3).

The FITC and the Fabp-eye-assay systems both have advantages and disadvantages when compared with each other. The advantage of the Fabp-eye-assay is that cardinal vein injection is not performed. This injection takes practice to be accurate, creates a danger to the fish such that it can be harmed or die in the process, and is even a great challenge in knockdown or overexpression studies that create severe edema. The advantages of the FITC-eye-assay are that it can be performed on any strain of fish where the FABP-eye- assay is dependent on the Fabp transgenic fish and that the genetically mutant fish can be screened [6].

We propose that, using the aforementioned techniques, it is possible to screen a large variety of genes in a short period of time and make solid statements regarding whether these genes are involved in the integrity of the glomerular filtration barrier. This screening system can be a helpful tool to discover novel genes, save time and resources and reduce the number of experimentally used rodents. Using these techniques, until now we were able to identify more than 25 novel genes formerly not described in glomerular biology (7).

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Nils Hanke, Lynne Staggs, Patricia Schroeder, Jennifer Litteral, Michaela Beese, Susanne Fleig, Jessica Kaufeld, Cornelius Pauli, Adina Agustian, Hermann Haller, Mario Schiffer

■ Projektleitung: Schiffer, Mario (Prof. Dr. med.), Haller, Hermann (Prof. Dr. med.); Förderung: MHH, MDIBL

Weitere Forschungsprojekte

Die Rolle des Endothels bei akuten und chronischen Nieren- und Gefäßerkrankungen

■ Projektleitung: Haller, Hermann (Prof. Dr. med.); Förderung: DFG, BMBF

A) Grundlagenwissenschaftlich: Mechanismen der Proteinurieentstehung B) Klinisch: Renale Co-Morbidität nach Transplantation solider Organe und des Knochenmarks

■ Projektleitung: Schiffer, Mario (Prof. Dr. med.); Kooperationspartner: Diverse MHH-interne, nationale und internationale Kooperationen; Förderung: DFG, SFB, IFB-Tx

Molekulare Schäden der Altersniere und fehlgeleitete Regenerationsmechanismen der Altersniere

■ Projektleitung: Schmitt, Roland (PD Dr. med.); Kooperationspartner: Diverse MHH-interne, nationale und internationale Kooperationen; Förderung: DFG, SFB 738, Fritz Thyssen-Stiftung

Regulation von Entzündungsprozessen bei Niereninsuffizienz

■ Projektleitung: von Vietinghoff, Sibylle (Dr. med.); Förderung: Intern, HiLF, MHH, DFG

Vaskuläre Biologie

■ Projektleitung: Doumler, Inna (Prof. Dr. rer.nat.); Förderung: BMBF, DFG, Else-Kröner-Fresenius-Stiftung

Nephrologische Intensivtherapie/Pharmakokinetik

■ Projektleitung: Kielstein, Jan (Prof. Dr. med.); Kooperationspartner: Diverse MHH-interne Kooperationen und nationale Kooperationen Magdeburg; Förderung: Caridian BCT Europe, Fresenius Medical Care

Nicht-invasive Diagnose der aktuten Rejektion bei Nierentransplantatatempfängern mittels Massenspektrometrie in Urinproben - Studie

■ Projektleitung: Gwinner, Wilfried (Prof. Dr. med.); Förderung: DFG, IFB-Tx

Akutes Nierenversagen und Transplantatabstoßung - experimentelles Mausmodell

■ Projektleitung: Güler, Faikah (Prof. Dr. med.); Förderung: BMBF

Chronisches Nierenversagen und Complement

■ Projektleitung: Güler, Faikah (Prof. Dr. med.); Kooperationspartner: Klos, Andreas (Prof. Dr.) Medizinische Mikrobiologie; Förderung: BMBF

Funktionelles MRT zur Charakterisierung von Nierenerkrankungen - klinische und experimentelle Untersuchungen

■ Projektleitung: Güler, Faikah (Prof. Dr. med.); Kooperationspartner: Hüper, Katja (Dr. med.) Radiologie; Förderung: DFG

Mechanismen der therapieresistenten Hypertonie

■ Projektleitung: Menne, Jan (PD Dr. med.); Förderung: BMBF

Mechanismen der akuten und chronischen Transplantatrejektion

■ Projektleitung: Einecke, Gunilla (PD Dr. med.); Förderung: SFB, IFB-Tx, Else-Kröner-Fresenius-Stiftung

Das Angiopoietin / Tie2 System im Kontext endothelialer Dysfunktion

■ Projektleitung: David, Sascha (Dr. med.); Förderung: DFG, Else-Kröner-Fresenius-Stiftung, Industrie

Myeloische Zelldifferenzierung in der ischämischen Neovaskularisierung: Regulation von Makrophagensubset durch die Notch Signaltransduktion

■ Projektleitung: Limbourg, Florian (Prof. Dr. med.); Förderung: DFG

Targeted delivery of Notch ligands for therapeutic arteriogenesis

■ Projektleitung: Limbourg, Florian (Prof. Dr. med.); Kooperationspartner: München, Heidelberg; Förderung: BMBF, DFG

Inflammation und kardiovaskuläre Erkrankungen bei Transplantationen

■ Projektleitung: Schmidt, Bernhard (PD Dr. med.); Förderung: ESAC, Wirtschaft

Vaskulitis und Nierenerkrankungen

■ Projektleitung: Wagner, Annette (Prof. Dr. med.); Förderung: Deutsches Stiftungszentrum, IFB-Tx, Wirtschaft

Originalpublikationen

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Beutel G, Kielstein JT, Ganser A. Thrombotische Mikroangiopathien. Internist (Berl) 2013;54(9):1070-1079

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Abstracts

2013 wurden keine Abstracts publiziert.

Habilitationen

David, Sascha Igor (PD Dr. med.): Das endotheliale Angiopoietin.

Schmitt Roland (PD Dr. med.): Mechanismen der epithelialen Tubulusschädigung und -reparatur im akutem Nierenversagen.

Promotionen

Denecke, Agnieszka (Dr. med.): Die Bedeutung von symmetrischem Dimethylarginin (SDMA) und asymmetrischem Dimethylginin (ADMA) als Biomarker bei Erwachsenen mit angeborenen Herzfehlern.

Dey, Hazra Emily (Dr. med.): Die Bestimmung zirkulierender Mikropartikel mit der Durchflusszytometrie: Einfluss unterschiedlicher Isolations- und Lagerungsprotokolle.

Egner, Nadine (Dr. med.): Telmisartan und Gefäßfunktion in diabetischen Patienten mit Hypertonie: Untersuchung zur Wirkung von Telmisartan auf Gefäßcompliance und Gefäßwiderstand.

Felix, Agnieszka Maria (Dr. med.): Phänotypisierung der CD73 Knockout Maus im Kontroll- und Glomerulonephritistier.

Förster, Katharina Angelika (Dr. med.): Der Einfluss präoperativer Serum-Harnsäurespiegel auf die Inzidenz eines akuten Nierenversagens nach Herzoperationen.

Ge, Shuwang (Dr. med.): Microparticle generation and leukocyte death in Shiga toxin mediated HUS.

Gerstein, Franziska (Dr. med.): Randomisierte prospektive klinische Studie zum Einfluss des Glucosegehaltes von Dialyselösungen auf den Langzeitverlauf bei Patienten nach Beginn der Peritonealdialyse.

Hodjat, Mahshid: Urokinase receptor in regulation of cellular senescence and DNA damage response: role for the ubiquitin-proteasomal system.

Jahr, Nicole (Dr. med.): Vergleich konventioneller vs. intensivierter Dialyse auf verschiedene Parameter der Organfunktion bei Intensivpatienten.

Susnik, Nathan David (Dr. rer. nat.): The role of proximal tubular suppressor of cytokine signalling 3 (socs-3) in acute kidney injury.

Veldink, Hendrik (Dr. med.): Symmetrisches Dimethylarginin (SDMA): Marker der akuten Nierenfunktionsänderung beim Menschen; Effekte der chronischen Infusion bei der C57Bl6J-Maus.

Wagner, Kathrin-Kristin (Dr. med.): Dauerhafte Blockade des Angiotensin II Rezeptors reduziert den (intra)renalen vaskulären Widerstand bei Patienten mit Diabetes mellitus Typ II.

Walter, Clemens (Dr. med.): Endotheliale Dysfunktion und arterielle Gefäßwandhypertrophie bei Patienten mit verminderter Glukosetoleranz.

Weitere Tätigkeiten in der Forschung

Haller, Hermann (Prof. Dr. med.): DFG-Fachkollegium Gutachter im Medizinausschuss des Wissenschaftsrats WR.

Schiffer, Mario (Prof. Dr. med.): Heisenberg Professur für Transplantationsnephrologie.

Kielstein, Jan (Prof. Dr. med.): Teilherausgeber NDT (Nephrology, Dialysis, Transplantation).