

## **A1 Günzel: Impedance analysis of epithelial barrier properties**

**Background:** Electrophysiological methods have a long tradition in epithelial physiology. However, new developments both in the molecular understanding of the tight junction (TJ) and in the measuring technology make new approaches possible.

**Hypotheses:** Combining impedance spectroscopy with specific alterations of partial epithelial resistances will allow us to quantify the contribution of different epithelial components towards general barrier function of simple and stratified epithelia. Refinement by combining brief, reversible alteration with fast multisinus impedance spectroscopy will generate a method for a detailed analysis of the mechanisms underlying the action of toxins and drugs.

**Aims:** This project aims to resolve the relative contributions of trans- and paracellular pathways as well as stratum corneum components towards barrier function in simple and stratified epithelia, respectively. Healthy and diseased tissues will be compared and effects of pharmacological treatment on different barrier components will be evaluated.

**Methods:** Standard molecular biological methods will be used for the knockout/knockdown of specific claudins and for the over-expression of ion channels or TJ components. Resulting cell clones will be cultivated (standard and 3D cell culture) and used for Ussing-chamber-based impedance spectroscopy. Multisinus technique for fast impedance spectroscopy has recently been implemented and will be rigorously tested in simple as well as stratified epithelial cell culture. Data will be evaluated by fitting to various equivalent circuit models and by using COMSOL Multiphysics® Modeling Software. Morphological conclusions from impedance measurements will be cross-validated with optical methods (confocal laserscanning microscopy; transmission electron microscopy).

**Thesis project:** As part of this PhD project we will adapt equivalent electric circuits by employing established cell culture models for simple and stratified epithelia. Expression of TJ proteins will be perturbed (e.g. by specific knock-down of TJ proteins or by application of cytokines). The transcellular pathway will be affected by application of ionophores, pore-forming bacterial toxins or by overexpression/activation of ion channels). Trans- and paracellular barrier components will be quantified by modelling impedance spectroscopic data, and visualized by various optical techniques.

**Requirements:** Enjoying Physics, Electronics and Informatics within the context of Biology.

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### **Suggested reading:**

Günzel D, Zakrzewski S, Schmid T, Pangalos M, Wiedenhoef J, Blasse C, Ozboda C, Krug SM (2012) From TER to trans- and paracellular resistance: Lessons from impedance spectroscopy. *Ann. N.Y. Acad. Sci.* 1257: 142-151

Schmid T, Bogdan M, Günzel D (2013) Discerning apical and basolateral properties of HT-29/B6 and IPEC-J2 cell layers by impedance spectroscopy, mathematical modeling and machine learning. *PLOS One* 8(7): e62913

Krug SM, Fromm M, Günzel D (2009) Two-path impedance spectroscopy for measuring paracellular and transcellular epithelial resistance. *Biophys. J.* 97(8): 2202-2211

## **A2 Piontek, Krause: Structure-based modification of *Clostridium perfringens* enterotoxin to target claudins**

**Background:** *Clostridium perfringens* enterotoxin (CPE) causes one of the most common food borne illnesses. CPE binds to tight junction (TJ) proteins (e.g. claudin-3 and -4) and subsequently forms pores in the membrane leading to damage of epithelial cells. Due to its cytotoxicity, CPE is used for targeting tumor cells that overexpress specific claudin-subtypes. In contrast, the isolated C-terminal domain of CPE (cCPE) is non-toxic and contains the claudin binding domain. cCPE is used as a TJ modulator to increase paracellular drug delivery. Previously, we elucidated in structure-based studies the molecular mechanism of the CPE-claudin interaction and designed CPE- and cCPE-variants with shifted subtype-specificity to claudins (e.g. binding to claudin-1 or -5 to which CPE-, cCPE-wt does not bind). We started to use these variants to (i) improve paracellular drug delivery in tissue barrier models, (ii) inhibit Hepatitis C Virus infection that depends on claudins and (iii) inhibit growth of thyroid and lung tumors growth.

**Aim** of this project is to expand structure-function studies to design and to test also new variants of cCPE and CPE that target further claudins. These proteins will be used as novel tissue-specific modular biologicals for (i) improved drug delivery by TJ modulation, (ii) molecular diagnosis or (iii) cytotoxic treatment of carcinomas.

**Methods:** Molecular modelling driven site-directed mutagenesis, expression and purification of recombinant proteins, protein-protein interaction assays, cell culture of differentiated epithelial and transformed carcinoma cells, immunocytochemistry and live cell confocal microscopy. Activity of cCPE- and CPE-variants will be tested by measurements of transepithelial resistance and paracellular solute permeability, imaging of cell lines, mouse and human tissue samples as well as cytotoxicity assays with cell lines and *in vivo* xenotransplant tumor models.

**Requirements:** Very good Master/Diploma degree in Biology, Biochemistry, Biotechnology or a related field of study, first experience in molecular cell biology and a strong interest in molecular / structural details of protein-protein interactions. We offer a strong, committed and enthusiastic scientific environment at the interface between basic and preclinical research.

**Contact:** joerg.piontek@charite.de, gkrause@fmp-berlin.de

### **Project-related publications:**

- Piontek A, Eichner M, Zwanziger D, Beier LS, Protze J, Walther W, Theurer S, Schmid KW, Führer-Sakel D, Piontek J, Krause G (2020) **Targeting claudin-overexpressing thyroid and lung cancer by modified *Clostridium perfringens* enterotoxin.** Mol Oncol. 14(2):261-276. doi: 10.1002/1878-0261.12615.
- Beier LS, Rossa J, Woodhouse S, Bergmann S, Kramer HB, Protze J, Eichner M, Piontek A, Vidal-Y-Sy S, Brandner JM, Krause G, Zitzmann N, Piontek J (2019) **Use of Modified *Clostridium perfringens* Enterotoxin Fragments for Claudin Targeting in Liver and Skin Cells.** Int J Mol Sci. 20(19). pii: E4774. doi: 10.3390/ijms20194774.
- Neuhaus W, Piontek A, Protze J, Eichner M, Mahringer A, Subileau EA, Lee IM, Schulzke JD, Krause G, Piontek J. (2018) **Reversible opening of the blood-brain barrier by claudin-5-binding variants of *Clostridium perfringens* enterotoxin's claudin-binding domain.** Biomaterials. 161:129-143. doi: 10.1016/j.biomaterials.2018.01.028.
- Eichner M, Augustin C, Fromm A, Piontek A, Walther W, Bückner R, Fromm M, Krause G, Schulzke JD, Günzel D, Piontek J (2017) **In colon epithelia, *Clostridium perfringens* enterotoxin causes focal leaks by targeting claudins which are apically accessible due to tight junction derangement.** J Infect Dis. 217(1):147-157. doi: 10.1093/infdis/jix485.
- Piontek A, Witte C, May Rose H, Eichner M, Protze J, Krause G, Piontek J, Schröder L (2017) **A cCPE-based xenon biosensor for magnetic resonance imaging of claudin-expressing cells.** Ann N Y Acad Sci. 1397(1):195-208. doi: 10.1111/nyas.13363.
- Eichner M, Protze J, Piontek A, Krause G, Piontek J. (2017) **Targeting and alteration of tight junctions by bacteria and their virulence factors such as *Clostridium perfringens* enterotoxin.** Pflügers Arch. 469(1):77-90. doi: 10.1007/s00424-016-1902-x. Review
- Protze J, Eichner M, Piontek A, Dinter S, Rossa J, Blecharz KG, Vajkoczy P, Piontek J, Krause G (2015) **Directed structural modification of *Clostridium perfringens* enterotoxin to enhance binding to claudin-5.** Cell. Mol. Life Sci. 72: 1417-1432
- Veshnyakova A, Piontek J, Protze J, Waziri N, Heise I, Krause G (2012) **Mechanism of CPE interaction with claudin-3/-4 protein suggests structural modifications of the toxin to target specific claudin.** J. Biol. Chem. 287: 1698-1708
- Walther W, Petkov S, Kuvardina ON, Aumann J, Kobelt D, Fichtner I, Lemm M, Piontek J, Blasig IE, Stein U, Schlag PM (2012) **Novel *Clostridium perfringens* enterotoxin suicide gene therapy for selective treatment of claudin-3 and -4 overexpressing tumors.** Gene Ther. 19: 494-503

### **A3 Fromm, Schulzke: Structural conditions of claudin-based water and cation channels**

**Background:** Our group has discovered that clusters of the tight junction (TJ) protein claudin-2 forms paracellular channels for small cations and also for water. In contrast, claudin-3 does not form channels but contributes to barrier function of the TJ. For the present project, we want to examine the interaction and tight junction strand distribution of claudin-2/claudin-2, claudin-3/claudin-3, and potential claudin-2/claudin-3 clusters. For this, we will generate hybrid claudin-2/claudin-3 cluster expressing cell lines and investigate their properties with regard to their paracellular water permeability using a proprietary method for measuring water transport in cell culture monolayers.

**Hypothesis:** In vector controls of cell cultures with high claudin-2 expression the TJ properties are mainly determined by the formation of "pure" pores based on claudin-2, which allow cations and water to pass. After claudin-3 transfection, a combined expression and interaction of claudin-2 and claudin-3 should predominate. Although claudin-2/claudin-3 hybrid pores could still be water-permeable they should have a reduced charge density within the pore. This would reduce cation permeability while maintaining water permeability.

**Work plan:** (A) Generation of stable epithelial cell clones with defined high or low claudin-2 expression as controls and additional defined expression rates of claudin-3. (B) Verification of the transfections and validation of the desired constancy of other claudins and aquaporins in the Western blot with densitometric evaluation. (C) Investigation of the localization and clustering of claudin-2 and -3 in an confocal and super-resolution microscope, in parallel to structure modeling studies performed in the partner project A2. (D) Freeze-fracture electron microscopy of tight junction strands of the different clones with subsequent morphometric evaluation. (E) Measurement of water permeability of the generated clones in comparison to the corresponding controls. The general goal of the project is to understand the structural prerequisites for claudin-based water and cation channels.

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**Project-related publications:**

- Amasheh S, Meiri N, Gitter AH, Schöneberg T, Mankertz J, Schulzke JD, Fromm M (2002) Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. *J. Cell Sci.* 115(24): 4969-4976
- Ayala-Torres C, Krug SM, Schulzke JD, Rosenthal R\*, Fromm M\* (\*shared last authorship) (2019) Tricellulin effect on paracellular water transport. *Int. J. Mol. Sci.* 20(22): e5700
- Milatz S, Krug SM, Rosenthal R, Günzel D, Müller D, Schulzke JD, Amasheh S\*, Fromm M\* (\*shared last authorship) (2010) Claudin-3 acts as a sealing component of the tight junction for ions of either charge and uncharged solutes. *Biochim. Biophys. Acta Biomembr.* 1798: 2048-2057
- Milatz S, Piontek J, Schulzke JD, Blasig IE, Fromm M, Günzel D (2015) Probing the cis-arrangement of prototype tight junction proteins claudin-1 and claudin-3. *Biochem. J.* 468(3): 449-458
- Rosenthal R, Milatz S, Krug SM, Oelrich B, Schulzke JD, Amasheh S, Günzel D, Fromm M (2010) Claudin-2, a component of the tight junction, forms a paracellular water channel. *J. Cell Sci.* 123(11): 1913-1921
- Rosenthal R, Günzel D, Krug SM, Schulzke JD, Fromm M, Yu ASL (2017) Claudin-2-mediated cation and water transport share a common pore. *Acta Physiol.* 219(2): 521-536
- Rosenthal R, Günzel D, Piontek J, Krug SM, Ayala-Torres C, Hempel C, Theune D, Fromm M (2020) Claudin-15 forms a water channel through the tight junction with distinct function compared to claudin-2. *Acta Physiol.* 228(1): e13334 (Editorial Commentary on this article: Alexander RT (2020) Claudin-15 is not a drag! *Acta Physiol.* 228(1): e13397)
- Yu ASL, Cheng MH, Angelow S, Günzel D, Kanzawa SA, Schneeberger EE, Fromm M, Coalson RD (2009) Molecular basis for cation selectivity in claudin-2-based paracellular pores: Identification of an electrostatic interaction site. *J. Gen. Physiol.* 133(1): 111-127

#### **A4 Haucke, Lehmann: Dynamic super-resolution imaging of tight junction components, lipids and ions**

The tight junction (TJ) connects neighboring epithelial or endothelial cells and acts as a barrier for solutes, water and pathogens. The TJ protein composition in tissues controls tightness and permeability and is regulated by dynamic protein assemblies, signaling and endocytosis.

The central aim of project A4 is to resolve the nanoscale molecular organization of TJs using super-resolution light microscopy and advanced live cell imaging under physiological and pathological conditions. We hypothesize that the nanoscale TJ strand composition and structure is changed by claudin/TAMP composition, toxin exposition, hypoxia and inflammatory responses. In order to understand organization principles and function of different TJ components we will combine STED microscopy with 50 nm resolution, FRET, FLIM, automated confocal microscopy and novel lipid/ion flux assays with quantitative image analysis. Specifically the dynamic nanoscale organization of all claudins, occludin, ZOs and selected pairs of claudins/occludin will be investigated in tissues, primary cells and CrispR knock-in and knock-out epithelial cell lines. All results will be used to understand disease, knockout phenotypes, pathological conditions and inspire new pharmacological treatments. Close collaborations with other basic and clinical research groups within TJ-Train are planned.

**Requirements:** Experience with Light Microscopy and Molecular Cell Biology, Computing skilly and previous experience with Image Processing are advantageous

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**B1 Willnow, Hammes: The endocytic receptor LRP2 serves as a central hub for dynamic cellular remodelling in neuronal stem cells and neural crest cells in the developing neural tube**

**Background and previous work:** The low density lipoprotein receptor related protein 2 (LRP2) is a multifunctional endocytic receptor localized at the apical surface of polarized epithelia. Patients with mutations in the *LRP2* gene suffer amongst other congenital anomalies from neural tube defects. LRP2 deficient mice consistently reflect disorders seen in humans. Our lab tries to understand the mechanisms underlying the neural tube closure defects caused by LRP2 loss of function.

Our recent results indicate towards a crucial role of LRP2 as a central hub in the periciliary compartment of neuroepithelial cells for establishing morphology, polarity and ultimately specification of neuronal progenitors. We showed that LRP2 functionally interacts with intracellular adaptor scaffold proteins and actin binding proteins that are involved in dynamic cell shape, apical constriction and planar cell polarity, processes that are crucial for neural tube closure. Moreover, we identified LRP2 in neural crest cells. Interestingly LRP2 deficient embryos show impaired cranial neural crest migration patterns suggesting an important role of the receptor not only in neuroepithelial cell specification but also in neural crest cell fate determination.

**Hypothesis:** LRP2 functionally interacts with intracellular cellular scaffolds and thereby serves as a hub to regulate single and collective cellular dynamics in polarized epithelia that involve rearrangements of the cytoskeleton and cell-cell junctions, including tight junctions.

**Aims, Work Plan and Methods:** At a cellular level we will try to understand the molecular and cell biological LRP2 dependent mechanisms responsible for neuroepithelial cell polarity and apical constriction, a prerequisite for proper neural tube formation. In addition, we will investigate the influence of LRP2 function on neural crest cell motility and cell fate decisions. In specific, we will address how the endocytic receptor LRP2 and its intracellular adaptor scaffold proteins affect cytoskeleton rearrangements and cell-cell connections and thereby confer neural tube formation as well as directed migration of neural crest cells.

Using mouse models and *ex vivo* explant cultures, we will employ various approaches based on molecular biology and imaging techniques. A strong focus will be on high and super resolution imaging to characterize details in the interaction of cellular components in neuroepithelial and neural crest cells. We are closely collaborating with project A4 (Martin Lehmann, Volker Haucke) optimizing super resolution imaging (STED) on whole mount embryos and explant cultures.

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**Project-related publications:**

Kur E, Mecklenburg N, Cabrera RM, Willnow TE, Hammes A. LRP2 mediates folate uptake in the developing neural tube. *J Cell Sci.* 2014 May 15;127(Pt 10):2261-8.

Christ A, Christa A, Kur E, Lioubinski O, Bachmann S, Willnow TE, Hammes A. LRP2 is an auxiliary SHH receptor required to condition the forebrain ventral midline for inductive signals. *Dev Cell.* 2012 Feb 14;22(2):268-78.

Gajera CR, Emich H, Lioubinski O, Christ A, Beckervordersandforth-Bonk R, Yoshikawa K, Bachmann S, Christensen EI, Götz M, Kempermann G, Peterson AS, Willnow TE, Hammes A. LRP2 in ependymal cells regulates BMP signaling in the adult neurogenic niche. *J Cell Sci.* 2010 Jun 1;123(Pt 11):1922-30.

## **B2 Schumann, Hummel: Epithelial barrier function secondary to defective epithelial polarity – a mechanism in celiac disease initiation?**

Celiac disease (CeID) is an enteropathy, in which an immune reaction leads to villous atrophy and crypt hyperplasia secondary to an infiltration of the small intestinal mucosa by T- and B-cells. Although immune pathology is activated by the wheat protein gliadin, the initiator of this genetically defined disorder is unknown. This is illustrated by the fact, that in some patients CeID initiation starts in early childhood, while in others it is not before the age of matured adulthood when CeID is about to start. One hypothetic cofactor for CeID initiation is the alteration of the mucosal barrier by colonization of the intestinal mucosa by either pathogenic or non-pathogenic bacteria.

Thus, this project aims to uncover the role of GI pathogens as causative for an epithelial barrier defect initiating CeID, which would then contribute to increased gliadin 'leakage' in the small intestinal mucosa, thereby pushing CeID immunity. In the previous B02 project, we have examined the role of two genes (LPP and C1orf106) in barrier function. These genes had been identified by the most recent whole genome association studies for celiac disease (Kumar et al., Human Molecular Genetics, 2015) and were shown to induce a defined barrier defect in intestinal epithelia.

Together with the collaborating Dept for Genetics in Groningen (Netherlands), we have manufactured intestinal epithelial CrispRCas9 knock-out cells. In the current project we plan to analyze the differential expression associated with the lack of LPP and C1orf106, respectively. Furthermore, we will colonize epithelial layers with various bacteria to induce a defect of epithelial polarity that might contribute to an increased uptake of gliadin peptides by the epithelial layer. This is determined in wild-type intestinal epithelia versus LPP and C1orf106-knock-out cells. Thereby, we can contribute to a better understanding of gliadin uptake in the pathogenesis of CeID.

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### **B3 Müller: Claudins of the thick ascending limb and nephrocalcinosis**

#### ***Project***

Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (FHHNC) is a deleterious rare autosomal-recessive renal tubular disorder caused by mutations in the *CLDN16* or *CLDN19* gene encoding the tight junction proteins Claudin-16 and Claudin-19. The disorder is characterized by renal wasting of calcium and magnesium and ultimately results in chronic kidney disease. Mouse models with deletion of the murine homologue *Cldn16* as already established by our group display hypomagnesemia and hypercalciuria but no nephrocalcinosis or renal insufficiency.

We hypothesize that lower urinary pH in mice is protecting the mice from nephrocalcinosis. Therefore, we will generate a mouse model that lacks additionally *Atp6v1b1*, a gene encoding a proton pump and involved in renal pH regulation. We will investigate if the elevated urinary pH will result in nephrocalcinosis. The resulting mouse model will help to understand the severe progression of the disorder in patients.

Mutations in *CLDN10* result in a multiplex epithelium disorder characterized by hypohidrosis, electrolyte imbalance, lacrimal gland dysfunction, ichthyosis, and xerostomia and designated 'HELIX syndrome'. Mice lacking claudin-10 specifically in the thick ascending limb display impaired renal salt handling reflected by hypermagnesemia und nephrocalcinosis. The nephrocalcinosis, which is not seen in patients, is presumably caused by calcium precipitation as consequence of a hyperresorption of calcium.

We aim at investigating the nature of the nephrocalcinosis as well as the precise localization. In combination, both models will help us to elucidate how hyper- and hyporesorption of divalent cations result in nephrocalcinosis.

#### ***Methods***

The deletion of *Atp6v1b1* will be achieved by CRISPR/Cas mediated genomic engineering in *Cldn16*<sup>-/-</sup> zygotes. The PhD student will perform a detailed biochemical as well as histological analysis of these mice. If present, the nephrocalcinosis will be analyzed by dispersive X-ray. This method will also be applied in the analysis of the claudin-10 deficient mice. Based on the results, the PhD student will develop strategies to ameliorate the nephrocalcinosis and influence thereby CKD.

#### ***Contact***

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## B4 Schmidt-Ott: Systems level assessment of epithelial barriers in the kidney

**Background:** The kidney tubule consists of different epithelial cell types (e. g. proximal tubule, distal tubule, collecting duct epithelial cells), which are characterized by marked differences in their paracellular transport characteristics and their tight junction composition. These epithelia and their barrier characteristics are important for central aspects of kidney function, including blood pressure control and osmoregulation.

**PhD project:** In this project we aim to comprehensively assess the molecular composition of epithelial barriers within cell types of the kidney using systems biology approaches. The goal will be to produce an atlas of tight junction component-encoding gene expression in the healthy and diseased kidney and to elucidate transcriptional networks associated with their gene expression control.

The project will focus on integration of multi-omics technologies, including single cell transcriptomics and spatially resolved transcriptomics, followed by focused validation experiments. A strong focus will be placed on bioinformatic integration of systems data. The successful candidate will work in an interdisciplinary environment including biologists, computer scientists, mathematicians and clinicians. Initial tasks will include setting up, applying and developing bioinformatics platforms and computational pipelines for the analysis of single cell and spatial transcriptomics and integrated downstream analyses.

The successful candidate will interact within a multidisciplinary environment of biologists, clinicians, computational scientists within the graduate school "TJ-Train" at Charité – Universitätsmedizin Berlin and at the Max-Delbrück Center for Molecular Medicine.

**Requirements:** Profound background in molecular biology, strong interest in computational data analysis, basic skills in programming (especially the R programming language and in scripting languages such as Perl/Python) are a plus.

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### Project-related publications:

1. Vukićević T\*, Hinze C\*, Baltzer S, Himmerkus N, Quintanova C, Zühlke K, Compton F, Ahlborn R, Dema A, Eichhorst J, Wiesner B, Bleich M, Schmidt-Ott KM\*\*, Klusmann E\*\*. Fluconazole Increases Osmotic Water Transport in Renal Collecting Duct through Effects on Aquaporin-2 Trafficking. *Journal of the American Society of Nephrology*. 2019, 30: 795-810. \*Co-first, \*\*Corresponding authors.
2. Vigolo E\*, Marko L\*, Hinze C, Müller DN\*\*, Schmidt-Ullrich R\*\*, Schmidt-Ott KM\*\*. Canonical BMP signaling in tubular cells mediates recovery after acute kidney injury. *Kidney International*, 2019, 95:108-122. \*Co-first, \*\*Equally contributing principal investigators.
3. Hinze C\*, Ruffert J\*(Co-first), Walentin K\*(Co-first), Himmerkus N, Nikpey E, Tenstad O, Wiig H, Mutig K, Yurtdas ZY, Klein JD, Sands JM, Branchi F, Schumann M, Bachmann S, Bleich M, Schmidt-Ott KM. GRHL2 is required for collecting duct epithelial barrier function and renal osmoregulation. *Journal of the American Society of Nephrology*. 2018, 29: 857-868.
4. Werth, M.\*, Schmidt-Ott, K.M.\* (co-first), Leete, T., Qiu, A., Hinze, C., Viltard, M., Paragas, N., Shawber, C.J., Yu, W., Lee, P., Chen, X., Sarkar, A., Mu, W., Rittenberg, A., Lin, C.S., Kitajewski, J., Al-Awqati, Q., Barasch, J. (2017). Transcription factor TFCP2L1 patterns cells in the mouse kidney collecting ducts. *eLife* 6, e24265.
5. Paragas N\*, Kulkarni R\*, Werth M\*, Schmidt-Ott KM\*, Forster C, Deng R, Zhang Q, Singer E, Klose AD, Shen TH, Francis KP, Ray S, Vijayakumar S, Seward S, Bovino ME, Xu K, Takabe Y, Amaral FE, Mohan S, Wax R, Corbin K, Sanna-Cherchi S, Mori K, Johnson L, Nickolas T, D'Agati V, Lin CS, Qiu A, Al-Awqati Q, Ratner AJ, Barasch J.  $\alpha$ -Intercalated cells defend the urinary system from bacterial infection. *Journal of Clinical Investigation*. 2014, 124:2963-76. \*Equal contribution.
6. Marko L\*, Vigolo E\*, Hinze C, Park JK, Roel G, Balogh A, Choi M, Wuebken A, Cording J, Blasig IE, Luft FC, Scheiderei C, Schmidt-Ott KM\*\*, Schmidt-Ullrich R\*\*, Muller DN\*\*. Tubular Epithelial NF- $\kappa$ B Activity Regulates Ischemic AKI. *Journal of the American Society of Nephrology*. 2016, 27:2658-69. \*Co-first, \*\*Equally contributing principal investigators.
7. Aue A, Hinze C, Walentin K, Ruffert J, Yurtdas ZY, Werth M, Chen W, Rabien A, Kilic E, Schulzke JD, Schumann M, Schmidt-Ott KM. A grainyhead-like 2/ovo-like 2 pathway regulates renal epithelial barrier function and lumen expansion. *Journal of the American Society of Nephrology*. 2015, 26:2704-15.
8. Walentin K, Hinze C, Werth M, Haase N, Varma S, Morell R, Aue A, Pötschke E, Warburton D, Qiu A, Barasch J, Purfürst B, Dieterich C, Popova E, Bader M, Dechend R, Staff AC, Yurtdas ZY, Kilic E, Schmidt-Ott KM. A Grhl2-dependent gene network controls trophoblast branching morphogenesis. *Development*. 2015, 142:1125-36.
9. Werth, M., Walentin, K., Aue, A., Schonheit, J., Wuebken, A., Pode-Shakked, N., Vilianovitch, L., Erdmann, B., Dekel, B., Bader, M., Barasch, J., Rosenbauser, F., Luft, F.C., and Schmidt-Ott, K.M. The transcription factor grainyhead-like 2 (Grhl2) regulates the molecular composition of the epithelial apical junctional complex. *Development*. 2010;137:3835-45.

## **C1 Schulzke, Troeger: Bacterial toxins' impact on epithelial tight junctions**

*Vibrio cholerae*, *Campylobacter concisus* and other pathogenic bacteria release zonula occludens toxins (ZOT) and ZOT-related toxins, which can affect the tight junction of gastrointestinal epithelia. These toxins are considered to cause diarrhea (leak flux diarrheal mechanism) and progression of inflammatory bowel diseases (leaky gut concept).

The doctoral student will study tight junction structure and function in cell and mouse models, organoids and human tissue specimens with molecular methods e.g. confocal microscopy and RNA sequencing (including pathway analysis) and with functional electrophysiological measurements. Effects of the toxins are studied by cloning and overexpression of toxins and are interpreted in comparison to the effects of whole bacteria and bacterial supernatants.

The central part of this project is concerned with extra- and intracellular signaling either directly at the epithelium or via the mucosal immune system, which is studied in co-cultures of epithelial cell models and/or organoids, together with M1-macrophages. Proinflammatory functionality is finally investigated using an inflammation-prone mouse model.

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## C2 Krug, Fromm: Interaction of immune cells and TJ proteins in IBD

**Background:** In inflammatory bowel diseases (IBD), the epithelial barrier is impaired, leading to loss of ions and water as well as enhanced luminal antigen uptake supporting inflammation. Alterations in expression and localization of tight junction (TJ) proteins are known to be involved in these processes. While claudins like claudin-2 are linked to increased paracellular ion and water flux, explaining the symptom of leak-flux diarrhoea, less is known about the basis of increased antigen uptake leading to immune response.

The tricellular TJ (tTJ) is considered to form a structural weak point of the barrier-forming TJ network and passage of antigens might be facilitated in this region in diseased states. Tricellulin, which is located at tTJs, plays a critical role for that as it is relevant for barrier formation against macromolecules and is affected in IBD.

**Hypothesis:** We hypothesize that besides being involved in luminal antigen uptake, the tTJ and its components may be also important in immune cell translocation and activation. It has been described that neutrophils and other leukocytes prefer tricellular corners for their passage and expression of tricellulin, but also of other TJ proteins, has been observed in cells of the monocyte/macrophage lineage. Based on these findings, we suggest an immunoregulatory role of TJ proteins and direct interaction between immune cells and TJs, especially tTJs.

**Project:** The doctoral thesis project we offer comprises characterizing the interaction of the (t)TJ barrier with subjacent immune-relevant cells and the mutual influence in developing IBD or inflammation in general. After isolating immune cells relevant for development and regulation of IBD and analyzing their potential expression profile of TJ proteins, direct interaction of the respective immune cells with their potential target TJ proteins will be visualized by live-cell confocal microscopy in cell lines expressing fluorescence-tagged TJ proteins. Interaction will be further characterized by higher resolved imaging techniques, by FRET (Förster resonance energy transfer), and by chemical coupling of the interacting partners and subsequent pulldown. The effect of immune response-activating substances will be analyzed to give insight into the regulation of tTJ permeability.

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### Project-related publications:

Krug SM, Bojarski C, Fromm A, Lee IM, Dames P, Richter JF, Turner JR, Fromm M\*, Schulzke JD\* (\*shared last authorship) (2018) Tricellulin is regulated via interleukin-13-receptor  $\alpha 2$ , affects macromolecule uptake, and is decreased in ulcerative colitis. *Mucosal Immunol.* 11(2): 345-356

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### **C3 Schumann, Sigmund: Loss of epithelial polarity as a mechanism in inflammation-induced carcinogenesis**

We have recently identified genes that are regulated in colitis-associated carcinogenesis (CAC), i.e. colon cancers that have progressed from either Crohn's disease (CD) or ulcerative colitis (UC, Sehn, Cardoso da Silva, et al. *in preparation*). Among the genes that were differentially expressed, osteopontin (OPN), a secreted glycoprotein expressed by various immune cells and epithelia, appears to be a master-regulator, inducing CAC via epithelial-to-mesenchymal transformation (EMT).

This highlights the role of genes that modulate the integrity of polarized epithelia and thereby either contribute to or prevent the development of carcinomas. The proposed project aims to uncover cell-biological mechanisms involved in OPN-induced EMT and carcinogenesis. So far, we have identified the MAP kinase pathway including downstream targets to be activated as well as excluded STAT3 and NFkappaB activation.

Within the future project we will analyze OPN's effects on enterocyte protein expression by RNAseq-analysis (human colon organoids) and changes in composition of polarity complexes, the epithelial barrier (including tight junctions) and the microtubule-associated cytoskeleton (3D cysts).

In collaboration with the pathology unit, another goal is to correlate the OPN-related expression signature with pre-carcinomatous (i.e. dysplastic) colonic lesions in CD and UC. Since another gene that we have identified to play a role in CAC induction is the transcription factor Grainy-head-like-2 (Grhl2), being implemented in EMT induction, the resumption of the already successful interaction with K. Schmidt-Ott's group is self-evident (Aue, J Am Soc Nephrol. 2015; Hinze, J Am Soc Nephrol. 2018).

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#### **C4 Epple, Schneider: Macromolecule uptake across the mucosa in HIV infection**

**Background:** Causing increased translocation of microbial components into the systemic circulation, a barrier defect of the gut epithelium is currently considered a key mechanisms of HIV immunopathogenesis. However, despite its assumed prominent role for HIV pathogenesis, an epithelial barrier defect for microbial macromolecules has not been characterized in the intestinal mucosa of HIV-infected individuals so far.

**Aims:** Combining clinical data with functional, molecular and immunological analyses of mucosal samples obtained from HIV-infected patients and HIV-negative controls and with data obtained in cell culture models, our project aims to characterize macromolecule translocation across the intestinal mucosa in HIV infection, define the route of macromolecule translocation, identify its trigger mechanism and compare small and large intestinal mucosa in these respects.

**Methods:** Methods employed will be: (i) Electrophysiological and transport physiological analysis of epithelial macromolecular transport and barrier function, (ii) immunofluorescence and confocal microscopy, (iii) cell culture, (iv), quantification of epithelial protein expression and apoptosis by standard assays, (v) subset analysis of mucosal immune cells and quantification of mucosal cytokine patterns by immunohistochemistry, flow cytometry, and cytometric bead array.

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