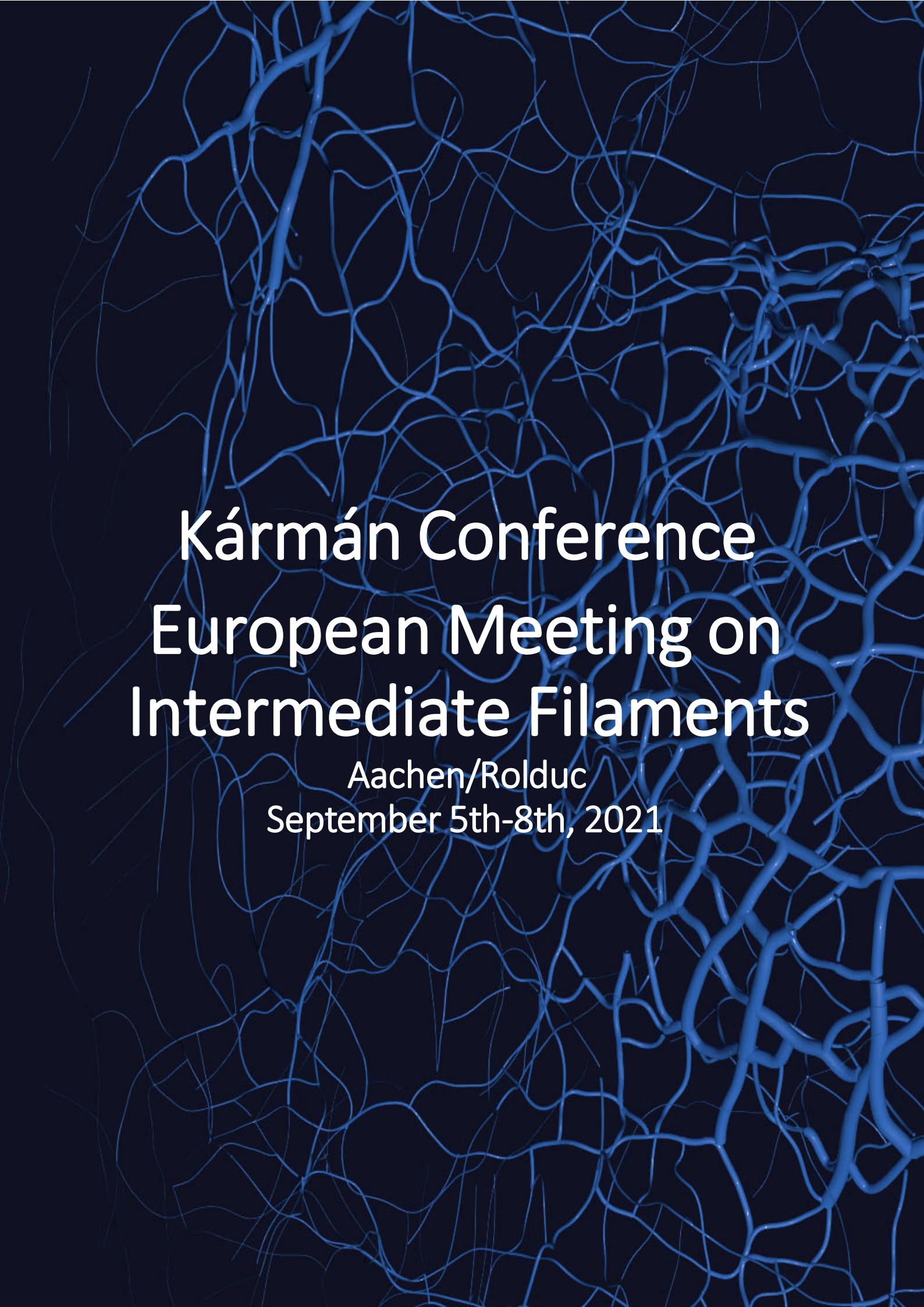

Kármán Conference
European Meeting on Intermediate Filaments

Aachen/Rolduc
September 5th-8th, 2021

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Table of contents

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In urgent cases we are available at 0049-176-85213027 during the conference.

Accommodation and Travelling

Venue

The conference will take place at Hotel Abdij Rolduc in Kerkrade (NL) in close vicinity to Aachen. Rolduc is the name of the largest preserved monastic complex in the Netherlands. Founded in the year 1104, it now serves as our conference center.

How to get there?

Aachen is Germany's westernmost city and is situated in the border zone between the Netherlands and Belgium. To the south, Aachen borders the Eifel and Hohes Venn.

The nearest airports are Cologne Bonn Airport (85 km), Düsseldorf Airport (99 km), Maastricht Aachen Airport (27 km) in the Netherlands, and Brussels Airport and Liège Airport (60 km) in Belgium.

Aachen main train station (Aachen Hauptbahnhof) is well connected to all airports.

The best option to travel from Aachen main train station to Rolduc is to take a cab (~ 35€). The cab stand is in front of the train station. Alternatively, you can take a train to Bahnhof Herzogenrath (~10 minutes). From there, it is a 5 minutes ride with a cab (~ 10€). We could also pick you up at Herzogenrath train station by car.

Accommodation

Accommodation and meals are included in the conference fee. We hope you will enjoy the comfortable rooms, delicious buffets as well as walking dinner and variety of drinks provided by Hotel Rolduc. Also, we have arranged fascinating activities for all the participants including an escape game and a Bajan music show with Victor Pribylov on September 7th. We sincerely wish you an unforgettable experience.

COVID-19

Participants must be vaccinated or tested negative for COVID-19. If you are coming from the EU, the EU digital covid vaccination certificate is sufficient. If you are coming from the US, the CDC Card is necessary. Please check the situation and the requirements shortly before your trip. You can also check our conference website for updates. Please bring enough FFP2 or medical masks with you. We will try to organize more masks and tests on Monday and Wednesday for people who are not vaccinated. The test center is near Rolduc (1 km away ~ 13 minutes walking). People who are not vaccinated will be informed separately. More information about the current rules can be found here https://www.germany.info/us-en/covid-19/2321562?openAccordionId=item-2468856-1-panel.

Please also check if you need a registration upon entering Germany at https://www.einreiseanmeldung.de and the regulations from the Netherlands can be found under https://www.government.nl/topics/coronavirus-covid-19/visiting-the-netherlands-from-abroad/checklist-entry.

Abbey Hotel Rolduc | Heyendallaan 82 | 6464 EP Kerkrade, Netherlands

Bajan Music with Victor Pribylov

Victor Pribylov grew up in Semipalatinsk (Kazakhstan, USSR) and began his musical education at the age of seven. He "fell in love" with the bayan instrument at a young age and since then he has been fascinated by this accordion-related instrument. The bayan is the Eastern European form of the chromatic button accordion and has a much larger range thanks to the buttons.

Escape Game

Somewhere on the scene a mysterious box is hidden. Where is it and what secret is kept inside? You will search in space for clues, riddles and puzzles. By solving them and completing tasks, you will find out the location of the mystery box. You have 90 minutes to find and open the Mystery Box. Which team will open the box first?

Keynote Speakers

Mike Boxem
Utrecht University

John T. Connelly
Queen Mary University of London

Pierre Coulombe
University of Michigan Medical School

Dennis Discher
University of Pennsylvania

John Eriksson
Turku Bioscience Center

Sandrine Etienne-Manneville
CNRS-Institut Pasteur

Roland Foisner
Medical University Vienna

Kathleen Green
Northwestern University

Harald Herrmann
University Hospital Erlangen

Elli Hol
University Medical Center Utrecht

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Leipzig University

Ohad Medalia
University of Zurich

Isabelle Migeotte
University Libre de Bruxelles

Milos Pekny
University of Gothenburg

Nicolas Plachta
University of Pennsylvania

Didier Stainier
Max Planck Institute for Heart and Lung Research

Sergei Strelkov
KU Leuven

Pavel Strnad
RWTH Aachen University

Abstracts – Keynote Lectures

Scaling concepts in ’omics:

lamin-B scales with tumor growth and predicts poor prognosis

Manasvita Vashisth[1,2], Sangkyun Cho[1,2], Jerome Irianto[1,2], Yuntao Xia[1,2], Mai Wang[1,2], Brandon Hayes[1,2], Farshid Jafarpour[1,3], Rebecca Wells[1], Andrea Liu[1,3], and Dennis E. Discher[1-3]*
[1]Physical Science Oncology Center at Penn, [2]Molecular & Cell Biophysics Laboratory, [3]Department of Physics/Graduate Group in Physics, University of Pennsylvania *discher@seas.upenn.edu

Abstract

Spatiotemporal relationships between genes expressed in tissues likely reflect physicochemical principles that range from stoichiometric interactions to co-organized fractals with characteristic scaling. For key structural factors within the nucleus and extracellular matrix (ECM), gene-gene power laws are found to be characteristic across dozens of tumor types in The Cancer Genome Atlas (TCGA) and also across single-cell RNA-seq data. The nuclear filament *LMNB1* scales with many tumor-elevated proliferation genes, including one transcriptional regulator FOXM1 that is confirmed experimentally. Although such cell cycle scaling and regulation is generally pro-survival across the TCGA tumor types, high *LMNB1* predicts poor survival of a patient within nine cancers including liver cancer. Also high in liver among other tumors are the main fibrosis-associated collagens, *COL1A1* and *COL1A2*, that scale with each other and with a pan-cancer fibrosis gene set. However, high fibrosis predicts prolonged survival of liver cancer patients, unlike *LMNB1*. Single-cell RNA-seq data also reveal scaling consistent with the pan-cancer power laws obtained from bulk tissue RNA and protein, allowing new power law relations to be predicted. Lastly, although noisy data frustrates weak scaling, concepts such as stoichiometric scaling highlight a simple, internal consistency check to qualify expression data.

Lamins regulate chromatin organization and gene expression through diverse mechanisms.

Simona Ferraioli, Fatih Sarigöl, Daria Filipczak, Nana Naetar and Roland Foisner

Max Perutz Labs, Medical University Vienna, Austria

Website: https://www.maxperutzlabs.ac.at/research/research-groups/foisner

A and B-type lamins form the nuclear lamina, a filamentous scaffold at the periphery of metazoan nuclei. Unlike B-type lamins, a pool of A-type lamins also localizes in the nuclear interior in a highly dynamic complex with the chromatin-binding LEM-protein lamin-associated polypeptide (LAP) 2alpha. While the peripheral nuclear lamina interacts with heterochromatic genomic regions, termed lamina-associated domains (LADs), contributing to stable gene repression, the dynamic lamin-LAP2alpha complex in the nuclear interior associates with euchromatic gene-rich regions outside of the LADs. We investigated lamin-LAP2alpha chromatin binding during in vitro myoblast differentiation in order to elucidate the physiological relevance and functions of chromatin-bound lamins and LAP2alpha. Both lamin A/C and LAP2alpha dynamically rearranged on chromatin during early stages of differentiation, and genomic regions bound by LAP2alpha and, to some extent also lamin A/C were enriched in differentially expressed genes. Deletion of LAP2alpha affected lamin A/C chromatin interaction and caused changes in muscle specific gene expression, probably due to decreased chromatin accessibility, thereby delaying muscle differentiation. At the gene level, LAP2alpha and lamin A/C were depleted at the promotor region of expressed genes but binding of lamins and LAP2alpha to the gene body did not correlate with gene expression changes. Altogether, our data suggest a novel role of nucleoplasmic lamins and LAP2alpha in the regulation of gene expression in a context- and cell typedependent manner by regulating genomic regions and chromatin environment rather than individual genes. This study was supported by the Austrian Science Fund (FWF).

Subnanometer structure of cellular vimentin filaments

Matthias Eibauer[1], Miriam S. Weber[1], Yagmur Turgay[1], Robert D. Goldman[2], and Ohad Medalia[1*]

[1]Department of Biochemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

[2]Department of Cell and Molecular Biology, Northwestern University Feinberg School of Medicine, 303 E Chicago Avenue, Chicago, Illinois, USA

Intermediate filaments are essential components of the cytoskeleton in metazoan cells. Due to their high mechanical flexibility they are principal contributors to elasticity and tear strength of cells and tissues. Vimentin, an intermediate filament protein expressed in fibroblasts and endothelial cells, assembles into 11 nm thick polymers, that are involved in a wide variety of cellular functions in health and disease. Here, we unveil the structure of cellularly polymerized vimentin filaments to a subnanometer resolution by applying cryo-electron tomography to detergent treated mouse embryonic fibroblasts grown on electron microscopy grids. We show that vimentin filaments are tube-like assemblies with a defined helical symmetry. Their structure comprises five octameric protofibrils and harbors 40 vimentin polypeptide chains in cross-section. In cells, vimentin displays two polymerization states characterized by the presence and absence of a luminal density along the helical axis. The implication of vimentin structure in light of previously published results, will be discussed.

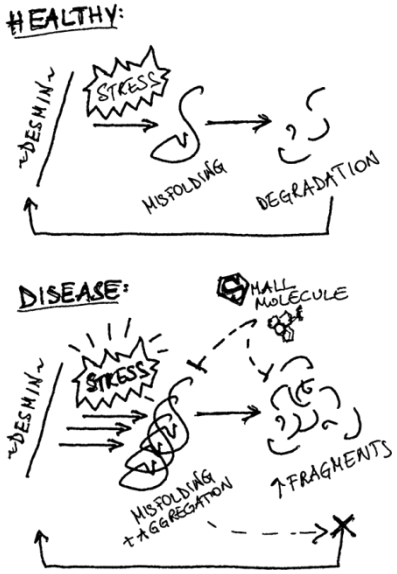
Abstracts – Selected Talks

Selected Talk – S01

"Targeting Desmin Loss of and Gain of Toxic Function in Chronic and Acute Heart Disease"

Pratima Rayavarapu[1], Stephen Carroll[1], Joseph Oldham[1], Krishna K. Singh[1], Giulio Agnetti[1,2]

[1]Center for Research on Cardiac Intermediate Filaments (CIRCF), Division of Cardiology and [2]Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, US



Introduction: Protein aggregation and fragmentation of both mutated and wild-type desmin protein sequences hallmark acute and chronic cardiac disease. Desmin cleavage and aggregation exacerbate disease by way of both loss of and gain of toxic function. With this in mind we set out to optimize a model to screen drugs that prevent either toxic mechanism or both, as well as addressing the causal role of desmin modifications in pre-clinical models.

Methods and Results: We optimized an *in vitro* model of acute, excessive desmin cleavage and aggregation, based on oxidative stress in neonatal rat ventricular myocytes (NRVMs). Oxidative stress is typically observed in cardiac disease of diverse origins and it is particularly relevant for the preventable reperfusion damage which follows myocardial ischemia, such as after a myocardial infarction. Using this model, we tested the potential protective effects of small molecules in terms of cell viability, desmin aggregation and cleavage, and other post-translational modifications (PTMs) that promote desmin gain of and/or loss of function. Based on the literature we selected three promising candidates: N-Acetyl Cysteine (NAC), epigallocatechin gallate (EGCG), geranyl-geranyl acetone (GGA). Cell cultures were treated with these small molecules either 3 hrs prior or 30' after treatment with 50 µM H_2O_2 to respectively address the potential molecular mechanisms of protection and transability (post-infraction) of these pharmacological interventions. Concurrently, we addressed the role of desmin PTMs (e.g. cleavage and oxidation) in in vivo and ex vivo models of ischemia/reperfusion (I/R) injury and ischemic preconditioning (IPC, an effective strategy that prevents I/R injury in vivo) to confirm the translational relevance of our in vitro observations.

Pretreatment with 1 mM NAC was able to significantly improve the reduction in full-length desmin levels induced by H_2O_2 in our in vitro model and reduce desmin cleavage, while use of or 50 µM GGA reduced desmin cleavage and aggregation. In addition, 20 µM EGCG was sufficient to significantly reduce desmin cleavage and increase the levels of full-length desmin. We also identified a novel, desmin-interacting protease by unbiased IP-MS. Intriguingly, the levels of this protease were increased with H_2O_2 treatment, along with desmin cleavage. Lastly, while desmin cleavage was reduced with IPC, mouse hearts coexpressing redox-dead desmin were more susceptible to I/R injury.

Conclusion: Collectively, our new preliminary data support the use of small molecules targeting desmin gain of and loss of function in the context of I/R injury and cardiac disease at large. In addition, we discovered a new cardiac protease which could be targeted pharmacologically to limit desmin cleavage.

Selected Talk – S02

Intermediate filament heterogeneity and its role in the mechanics of glioblastoma invasion

Emma van Bodegraven[1,2], Florent Peglion[1], Sandrine Etienne-Manneville[1] [1]

Cell Polarity, Migration and Cancer Unit, Institut Pasteur, UMR3691 CNRS, Equipe Labellisée Ligue Contrele Cancer, Paris, France [2] *Department of Translational neuroscience, Brain Center University Medical Center Utrecht, Utrecht University, The Netherlands*

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Glioblastoma multiforme (GBM) is the most common malignant brain tumor with a poor prognosis and no curative therapy. GBM invasion largely contributes to the poor prognosis and therapeutic failure. Knowledge on the mechanism of GBM cell invasion is essential to develop new treatments. Recent literature points towards the mechanical properties of tumor cells and the extracellular matrix as crucial players in GBM invasion. Because of their inherent physical properties and crosstalk with other cytoskeletal elements, intermediate filaments (IFs) significantly impact cell mechanics. This study aims to determine the contribution of IFs in the mechanics of GBM cells and GBM invasion.

Glial and GBM cells express the IF proteins GFAP, Vimentin, Nestin, and Synemin. GBMs are highly heterogeneous tumors which is likely to be reflected by a large diversity in the expression of these IF genes. To map the heterogeneity of IF expression in GBMs, we have analyzed published single-cell RNA sequencing data generated from patient material. Hierarchical clustering of single GBM cells based on IF gene expression identified 12 different cell clusters and showed that high IF gene expression signatures associate with markers of cells localized at the tumor periphery. We further observed a strong correlation between high IF gene expression and cell migration and mechanosensing gene ontology clusters.

Using CRISPR-Cas9 technology to alter IF gene expression in GBM cell models, we recapitulated the association between IF expression and GBM invasion-related gene expression. In 3D in vitro and in vivo migration assays, we have observed that the loss of IF expression decreases the ability of GBM cells to invade as leaders and impairs in vivo invasion in the zebrafish brain. We now use 2D and 3D migration assays on different rigidities and confined environments to further unravel how IFs modulate cell mechanics to promote GBM invasion.

Selected Talk – S03

The role of A-type lamins in Bone Development

Claudia Hufnagel, Cindy Simon, Silvia Spitzer, Norbert Hassler, Jochen Zwerina and Thomas Dechat*

Ludwig Boltzmann Institute of Osteology at Hanusch Hospital of OEGK and AUVA Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Vienna, Austria; *presenting author

Nuclear lamins, type-V intermediate filament proteins, are the main constituents of the nuclear lamina, a filamentous meshwork underlying the inner nuclear membrane. They are divided into A- and B-types. A-type lamins, with lamins A and C as major isoforms, are derived from a single gene, *LMNA*, by alternative splicing. Besides providing shape and stability to the nucleus, lamins are involved in several cellular processes such as cell proliferation and differentiation, mechanosensing and –signalling, gene expression and chromatin organization. Furthermore, over 500 mutations in *LMNA* have been associated with several human diseases including muscular dystrophies, cardiomyopathies, lipodystrophies and premature aging. Some of these mutations, especially those linked to accelerated aging, display also a bone phenotype. Also recent studies have shown that knock-down of lamins A/C by siRNA in human mesenchymal stem cells and in human osteoblasts leads to impaired osteoblast differentiation. In addition, over-expression of lamin A in the murine pre-osteoblast cell line MC3T3-E1 leads to accelerated differentiation into mature osteoblasts. To study the role of A-type lamins in bone development in general and specifically during the course of osteoblast differentiation we generated MC3T3-E1 as well as murine bone marrow derived stromal D1 cell clones deficient in A-type lamins using the CRISPR/Cas9 method. As expected, MC3T3-E1 as well as D1 cell clones lacking lamins A/C display misshapen and lobulated nuclei. In addition, cell proliferation is delayed. When induced to differentiate into osteoblast, MC3T3-E1 as well as D1 cell clones displayed impaired differentiation and mineral deposition potential as revealed by alizarin red staining and determining alkaline phosphatase activity. In addition, expression of collagen 1a was delayed and decreased as compared to wild type cells, suggesting that the generation of the extracellular matrix (ECM), which is a major step in osteoblast differentiation and also generally in bone synthesis, was impaired. A delayed/decreased ECM formation was also observed in the MC3T3E1 cell clones by attenuated total reflection-Fourier transformed infrared spectroscopy. Since extracellular vesicles (EVs) have also been shown to play a role in bone development we isolated EVs from D1 cell clones and wild type cells at different days during osteoblast differentiation.

Initial analysis suggests that the EV production was decreased when A-type lamins were lacking. Summing up we can show that lamins play a role during ECM generation and EV production occurring during osteoblast differentiation. Further analysis of the EVs by mass spectroscopy and comparing the expression profiles of wild type and knock out cells during the course of osteoblastogenesis will shed more light on the role of A-type lamins in bone development.

Selected Talk – S04

Perturbed intermediate filament regulation causes aggregate toxicity

Authors:
Florian Geisler [1]; Sanne Remmelzwaal [2]; Christine Richardson [3]; Mike Boxem [2]; Rudolf Leube [1]

Affiliations:
1) Institute of Molecular and Cellular Anatomy, RWTH Aachen University, Aachen, Germany; 2) Institute of Biodynamics and Biocomplexity, Utrecht University, Utrecht, The Netherlands; 3) School of Biological and Biomedical Sciences, Durham University, Durham, United Kingdom

Email: fgeisler@ukaachen.de

Intermediate filaments are major components of the metazoan cytoskeleton. A longstanding debate concerns the question whether intermediate filament network organization only reflects or also determines cell and tissue function and dysfunction. This is particularly relevant for aggregate-forming diseases involving intermediate filaments. Using *C. elegans* as a genetic model organism, we have recently described mutants of signaling and stress response pathways with perturbed intermediate filament network organization. In a mutagenesis screen, we now identify the intermediate filament polypeptide IFB-2 as a highly efficient suppressor of these phenotypes restoring not only intestinal morphology but also rescuing compromised development, growth, reproduction and stress resilience. Ultrastructural analyses show that downregulation of IFB-2 leads to depletion of the aggregated intermediate filaments. The findings provide compelling evidence for the toxic function of deranged intermediate filaments and reveal novel insights into the cross talk between signaling and structural functions of the intermediate filament cytoskeleton.

Selected Talk – S05

Title: Severe structural and mitochondrial dysfunctions caused by a heterozygous *DES* mutation in iPSC-derived cardiomyocytes

Authors: Yeranuhi Hovhannisyan[1], Pierre Joanne[1], Dorota Jeziorowska[1], Alexandre Simon[1], Gaëlle Revet[1], Coline Rogue[1], Jocelyne Blanc[1], Ekaterini Kordeli[1], Paul Fornes[2,3], Domitille Callon[2,3], Hakim Hocini[4], Jean-Paul Concordet[5], Gérard Tachdjian[6], Anthony Béhin[7], Karim Wahbi[8], Zhenlin Li[1] & Onnik Agbulut[1].

Affiliations : [1] Sorbonne Université, Institut de Biologie Paris-Seine (IBPS), CNRS UMR 8256, Inserm ERL U1164, Biological Adaptation and Ageing, 75005 Paris, France. [2]Laboratoire de biopathologie, hôpital Robert Debré, institut-médico-légal, CHU, avenue du Général Koenig, 51100 Reims, France. [3]EA-4684, CardioVir, Université de Reims Champagne Ardenne, 51100 Reims, France. [4]INSERM, U955, Team 16, Clinical and Infectious Diseases Department, Hospital Henri Mondor, University of Paris East, Créteil,
France. [5]Laboratoire Structure et Instabilité des Génomes, Inserm U1154, CNRS UMR 7196, Museum National d'Histoire Naturelle, Paris, France. [6]Laboratoire de Cytogénétique, Service d'HistologieEmbryologie-Cytogénétique, Hôpital Antoine Béclère, AP-HP, Université Paris Saclay, Clamart, France. [7]AP-HP, Pitié-Salpêtrière Hospital, Reference Center for Muscle Diseases Paris-Est, Myology Institute, Paris, France. [8]AP-HP, Cochin Hospital, Cardiology Department, Paris, France.

Objective: Mutations of desmin, the major intermediate filament of muscle cells, often lead to skeletal and cardiac myopathies. Several mutations are linked to the development of dilated cardiomyopathy (DCM). The purpose of this study was to investigate the role of human desmin heterozygous mutation of E439K on the structural organization and function of cardiomyocytes.

Methods: For this purpose, induced pluripotent stem cells (hiPSC) were generated from patient blood cells using non-integrative reprogramming method. Patient-specific hiPSCs were successfully differentiated into contractile cardiomyocytes (CMs) and structurally and functionally characterized. Results obtained on these patient lines were further validated using an isogenic pair that was created by CRISPR/Cas9-mediated base editing and were compared with cardiac biopsies from a patient harboring the *DES*^{E439K} mutation.

Results: Immunofluorescent studies demonstrated alterations in the cytoarchitecture of E439KCMs compared to Control-CMs including the organization of the M-line and I-band of the sarcomere as detected by cardiac Troponin T immunostaining. We also provided evidence for a dramatic change in overall cardiomyocyte size and morphology of E439K-CMs. Mitochondrial dysfunction is very often observed in the skeletal muscle of patients with myofibrillar myopathies and also in patients affected by heart failure. In this study, we also found that E439K-CMs showed decreased mitochondrial respiration compared to ControlCMs. Mitochondrial abnormalities were also analyzed by immunostaining, western-blot and electronic microscopy.

Conclusion: Taken together, our results confirm that *DES* mutations can cause structural and functional defects in human cardiomyocytes. Most importantly, the structural changes in mitochondria and a dramatic decrease in mitochondrial activity are for the first time, demonstrated in a human model of DCM induced by a *DES* mutation. As a consequence, different methods to improve mitochondrial respiration are currently being assessed to try to revert the diseased phenotype of E439K-CMs and evaluate their therapeutic potential.

Selected Talk – S06

Engineering an artificial plectin to understand the interaction of actin and vimentin

Irene Istúriz, Zima Kabir, Gijsje Koenderink

TU Delft

Cytoskeletal crosstalk plays an essential role in the balance between deformability and strength in the cells. In order to unravel the mechanical synergy emerging from cytoskeletal crosstalk we study reconstituted cytoskeletal composite networks. In this particular study we focus in the interaction of actin and vimentin and aim to directly mediate this interaction by crosslinking and understand the properties emerging and arising from this interaction. We are engineering a library of artificial crosslinkers, focusing on plectin. In this study we present an engineered artificial plectin, which we are testing to reveal whether we can directly mediate an interaction between actin and vimentin. This engineered plectin contains an actin binding domain and a vimentin binding domain, separated by a coiled coil. Moreover, it includes a domain of eGFP. Preliminary results show that the crosslinker appears to be functional and diverse mechanical and reorganization properties appear.

Selected Talk – S07

Vimentin intermediate filament networks are required for compression stiffening of cells and protection of nuclei from compressive stress.

Katarzyna Pogoda[1], Alison A Patteson[2], Paul A. Janmey[3]

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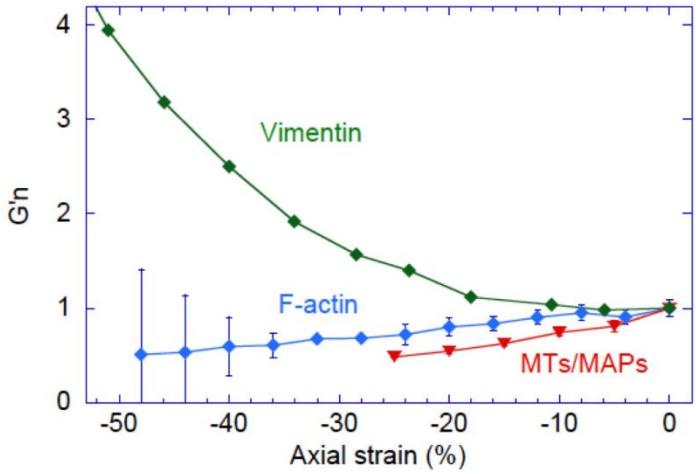
2. Physics Department and BioInspired Institute, Syracuse University, Syracuse, NY 13244
3. Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, PA 19104

Abstract

Rigid and semi-flexible biopolymer networks generally stiffen when deformed in shear but soften in compression due to filament buckling. However, unlike crosslinked networks of purified F-actin or microtubules, which soften in compression, vimentin intermediate filament (VIF) networks stiffen in both compression and extension by a mechanism that involves the greater flexibility of VIFs and the large surface charge of the vimentin filament that resists volume changes under compression. Enforcing local and global volume conservation shifts buckling modes of the filaments, which soften the network, into stretching modes, which stiffen it. Individual cells, such as fibroblasts, stiffen at physiologically relevant compressive strains, but deletion of vimentin diminishes this effect. Lack of VIFs in vimentin null fibroblasts leads to greater damage to the nucleus after cell compression. These results provide a new framework by which to understand the mechanical responses of cells and point to a central role of intermediate filaments in response to compression



Selected Talk – S08

Inflammatory activation and metabolic impairment, are important mediators of desmin related cardiomyopathy

E. Mouchtouri1[1], Z. Kotsaridou[1], A. Varela[2], I. Kostavasili[1], C. Davos[2], C.D. Anagnostopoulos[2] and M. Mavroidis[1]*

[1] Basic Research and [2] Clinical, Experimental Surgery, & Translational Research, Biomedical Research Foundation, Academy of Athens, Athens Greece.

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Introduction: Inflammatory activation and metabolic impairment are becoming the focus of research as novel therapeutic targets in heart failure. We have demonstrated in a genetic model of arrhythmogenic cardiomyopathy (desmin-deficient mice, Des-/-) that modulation of innate immunity through elimination of complement C5a receptor (C5aR) resulted in impressive improvement of cardiac function.

Aim: To analyze the role of the second C5a receptor (C5L2) that has been linked to energy metabolism and inflammation as a novel therapeutic target in desmin related cardiomyopathy.

Materials/Methods: We generated C5L2-/-Des+/- mice by crossing C5L2-/- with Des-/- mice. Histology, electron microscopy, echocardiography, RNAseq and ^{18}F-FDG microPET/CT were performed in 12 months old animals (n=10) and parameters related to cardiac structure, function and myocardial glucose consumption were compared with those of wild type (WT) controls of similar age.

Results: C5L2-/-Des+/- mice progressively developed severe cardiac dysfunction compared to Des+/- or WT controls (Fractional shortening 22.89±2.52 vs. 46.94±0.67, $p<0.0001$). Histology revealed increased fibrosis in C5L2-/-Des+/- compared to WT (Fibrosis index, 1.5±0.21, vs. 0.4±0.34, $p<0.01$). Electron microscopy showed severe mitochondrial and T-tubules abnormalities in C5L2-/-Des+/- compared to WT. Additionally, cardiac tissue RNAseq analysis demonstrated altered expression of several genes involved in metabolic pathways, indicating a "metabolic switch" in C5L2-/-Des+/- from fatty acid to glucose oxidation compared to WT. This was also confirmed by the higher myocardial metabolic rate of glucose values in C5L2-/-Des+/- compared to WT animals (168.6 ±55.2 vs. 39.8±3.3µmol/min/100g, $p<0.05$).

Conclusions: Our results highlight the detrimental consequences on cardiac structure and function of C5L2 receptor elimination in desmin related cardiomyopathy and support the hypothesis of its implication in the metabolic impairment, which occurs in this pathological entity.

Selected Talk – S09

Identification of a novel function of desmoglein 2 as a suppressor of hematopoiesis in the embryonic mouse heart

Hoda Moazzen, Kateryna Venger, Sebastian Kant, Rudolf E. Leube, Claudia A. Krusche

Institute of Molecular and Cellular Anatomy, RWTH Aachen University, Wendlingweg 2, 52074 Aachen, Germany

Abstract

The desmosome-specific cadherin desmoglein-2 (Dsg2) supports adhesive strength between cardiomyocytes to withstand the mechanical stress imposed by the life-long cardiac contraction cycles. Consistent with this notion, *Dsg2*-mutant mice develop excessive chamber dilation mimicking human arrhythmogenic cardiomyopathy that has been linked to *Dsg2* mutations. On the other hand, functions of Dsg2 for cardiac morphogenesis have not been explored despite the reported mid-gestational mortality of murine *Dsg2* mutants. We now show that cardiogenesis is perturbed in >70% of *Dsg2*-mutant embryos frequently resulting in pericardial hemorrhage and lethal myocardial rupture. On close inspection, mutant hearts present two types of abnormal cell clusters: Type A cell clusters involve endocard-associated roundshaped $CD31^+$ cells, which proliferate and invade the myocardium. The cell clusters acquire Runx1- and CD44-positivity indicating a shift towards a hematopoietic phenotype. Type B cell clusters appear next to type A clusters and expand in the subepicardium forming large spheroids. Type B cell clusters consist primarily of $Ter119^+$ erythroid cells with interspersed $Runx1^+/CD44^+$ cells suggesting that they originate from type A cell clusters. The observed pericardial hemorrhage is caused by migration of erythrocytes from type B cell clusters through the epicardium and rupture of the altered cardiac wall. Finally, evidence is presented that the observed pathogenesis is secondary to structural defects of Dsg2-depleted cardiomyocytes inducing paracrine signaling, which involves Notch1. Taken together, our observations uncover a hitherto unknown regulation of hematopoiesis in the embryonic heart by Dsg2.

Selected Talk – S10

Vimentin regulates cell growth through the mTORC1 signaling pathway

Leila S. Coelho Rato[1,2]*, Mayank Modi[1,2], Peter James[1,2,3], John E. Eriksson[1,2,+]

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Recently we found an important role for the intermediate filament vimentin in cell growth regulation. Briefly, we showed that, due to lower activation of mTORC1, fibroblasts lacking vimentin had lower levels of protein synthesis and higher levels of autophagy, resulting in a significantly smaller cell size. However, the molecular mechanism behind this phenomenon remains unknown. To characterize in greater detail the involvement of vimentin in this process, we used proteomics examine which vimentin-interacting signaling partners are to be found under starved versus growth stimuli. Moreover, we performed phosphoproteomics analysis to access the changes in the phosphoproteome under these conditions. With this approach we have identified several new vimentin-interacting partners which could explain the observed differences in mTORC1 signaling. We could also identify clear switches in the phosphostate of proteins involved in cell growth and proliferation signaling, which are consistent with our hypothesis. Together, these results provide new insights on vimentin-mediated growth signaling, and thus improve our understanding on how cells adjust to nutrient-deprived environments, such as in wound healing and cancer.

Selected Talk – S11

aPKC regulates keratin-dependent mechanical tissue resilience

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Abstract

Epithelia need dynamic cell rearrangement to self-renew while also maintaining tissue integrity to guarantee barrier function in the face of external stress. The cadherin-based intercellular adhesive adherens junctions (AJ) and desmosomes are essential for tissue cohesion. Whereas the actin-linked AJ enable cell rearrangement, keratin filaments interact with desmosomes to strengthen mechanically challenged tissue like the heart or skin. How cells regulate resilience and junctional reorganization to maintain tissue integrity during homeostatic, renewal or adapt to mechanical is stress is not known. To address this question, we use primary multilayered keratinocytes as a paradigm for the stratified skin epidermis. We identify the polarity protein atypical kinase C, (aPKC) as a key determinant of not only basal AJ and desmosomes, but also of the suprabasal resilience state in stratified keratinocytes. Using sheet dissociation assays and tissue-stretching assays we find that loss of aPKC promotes sheet resilience of stratified sheets but not of basal cells. Interestingly, although desmosomes are essential for suprabasal integrity, this was not accompanied by a change in their number and ultrastructure upon loss of aPKC. Instead, aPKC loss alters suprabasal keratin organization in vivo and in vitro into a more filamentous stressed network. This change is accompanied by a reorganization of cortical F-actin into stress fibers, indicative of increased actomyosin activity. Importantly, this increase controls keratin organization as inhibiting contractility in aPKC$^{-/-}$ cells reversed keratin organization and lowered resilience, whereas activation of myosin activity in control cells was sufficient to induce stressed keratins and increased resilience. In conclusion, our data suggest a model in which aPKC serves as a mechanical rheostat that adapts actomyosin activity to control suprabasal keratin organization and tissue resilience while coordinating junctional reorganization of basal cells, thus promoting stratification while maintaining integrity.

Selected Talk – S12

Multiscale mechanics of reconstituted vimentin networks

A. V. Schepers [1,2], C. Lorenz [1], P. Nietmann [3], A. Janshoff [2,3], S. Klumpp [2,4], S. Köster [1,2]

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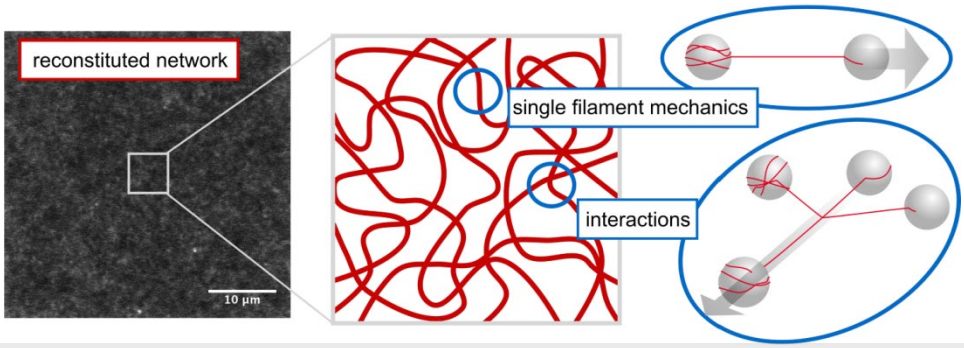
Among the three main filamentous components of the cytoskeleton, the intermediate filament (IF) network is by far the most extensible and resilient to stress. We present a multiscale approach to



Disentangling the mechanical contributions of single filament properties and interactions to network mechanics .

disentangle the three main contributions to reconstituted vimentin IF network mechanics — single filament mechanics, filament length, and interactions between filaments — including their temporal evolution. Combining particle tracking, quadruple optical trapping and computational modeling, we derive quantitative information on the strength and kinetics of filament interactions. Specifically, we find that hydrophobic contributions to network mechanics enter mostly via filament elongation kinetics, whereas electrostatics has a direct influence on filament—filament interactions. These results indicate that cells might need to explicitly suppress attractive interactions to re-organize the extremely stable cellular vimentin network.

Selected Talk – S13

Title: Role of lamin-mediated nuclear deformability in cancer invasion and metastasis *in vivo*

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Introduction: Distant metastasis of solid tumors, such as in melanoma or sarcoma, represents a significant clinical challenge. Critical steps during tumor progression, such as cell invasion through the tumor microenvironment, transport in the blood stream and extravasation into secondary organs depend on the mechanical stability and deformability of tumor cells. The cell nucleus limits cell deformation during invasion, reduces the efficacy of tumor cell motility in dense tissue, and further can undergo nuclear envelope rupture and DNA damage. Lamins A and C form part of the nuclear envelope, interact with both chromatin and the cytoskeleton, and represent important contributors to the viscoelasticity of the nucleus which impacts cell migration efficacy *in vitro*. Lamins are frequently deregulated in various cancer types, but their contribution to cancer invasion and metastasis *in vivo* remains unclear.

Objective: The goal of this project is to investigate the impact of lamin regulation on cancer cell invasion, loss of nuclear envelope integrity and DNA damage incidence, as well as metastasis in a dermis-based mouse *in vivo* model. Therefore, stable lamin-expression modified HT1080 human fibrosarcoma, MV3 human melanoma, and B16F10 mouse melanoma cell lines were generated. The engineered tumor cells were implanted into a living mouse, to examine invasiveness and metastasis formation in a preclinical setting.

Methods and Results: Using CRISPR/Cas9 technique, cell transduction, cell sorting, and clonal selection, several clonal tumor cell lines were generated that stably express a nuclear localization sequence coupled to copGFP (NLS-copGFP) serving as a nuclear envelope integrity indicator, a DNA damage repair sensor $53BP1_{trunc}$-mApple, and a H2B-mVenus fusion protein allowing simultaneous use of lamin-expression modified and control cell lines. Clonal lamin-depleted cell lines showed higher nuclear irregularity increased migration efficacy and nuclear envelope rupture incidence in confining 3D environments. Preliminary i*n vivo* experiments showed viable cells that migrated within the mouse dermis, together with the detection of nuclear leakage and DNA damage. In addition, lamin depletion correlated with decrease in surface lung metastasis formation.

Conclusion: Several functional clonal lamin A/C knockout tumor cell lines, expressing reporters of nuclear envelope leakage and DNA damage repair were generated and characterized. Ongoing *in vivo* studies will provide insight into the requirement for lamin A/C expression regulation on tumor cell adaptability in heterogeneous tissue structures, nuclear integrity, DNA damage rate, cell survival and metastatic load in the living organism.

Selected Talk – S14

Intestinal epithelial keratin 8 is essential for colonic tissue integrity and protection from colon tumorigenesis

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Keratins are epithelial intermediate filament proteins responsible for cell stress protection and maintenance of cell polarity. Mutations in keratin have been linked to several human diseases in skin, liver and possibly intestine. Many of these diseases are phenocopied in keratin transgenic and knockout mice, and the full keratin 8 knockout ($K8^{-/-}$) mouse suffer from liver fragility, inflammation of the colon, colonic hyperproliferation, altered ion transport, diarrhoea, and alterations in insulin secretion. Because these mice have severe problems in most simple epithelial organs where K8 is expressed, it is unclear whether the colon phenotype is caused by keratin loss primarily in colonic epithelial cells, or as a secondary effect triggered by e.g. defects in the liver or pancreas. To answer this question, we created a conditional $K8^{-/-}$ mouse, with intestine specific downregulation of K8 by using the Cre-loxP system. $K8^{flox/flox}$ mice were bred with Villin-Cre mice (both tamoxifen inducible and ubiquitous Cre expressing mice were used), which ultimately yielded $K8^{flox/flox}$;VillinCre mice. K8 deletion in both conditional knockout models decreased the type II K8 partner proteins, type I K18-K20, with 75-95 %. The remaining keratin filaments were located to the colonocyte apical regions with type II K7, that decreased 30 %. [^{18}F]FDG *in vivo* PET imaging identified a metabolic phenotype in the lower gut of conditional germline K8-knockouts. These mice developed intestinal barrier leakiness, mild diarrhoea, and wide epithelial damage, especially in the proximal colon. Mice exhibited a shifted differentiation from enterocytes to goblet cells, displayed two-fold longer crypts and an increased number of Ki67+ transit amplifying cells in the colon. Significant pro-proliferative and regenerative signalling occurred in the IL-22, STAT3, and pRB pathways, with minor effects on inflammatory parameters, which, however, increased in ageing mice. In addition, colonocyte K8 deletion induced a dramatically increased sensitivity to azoxymethane-induced tumorigenesis. In conclusion, intestinal epithelial K8 plays a significant role for colonocyte epithelial maintenance, proliferation regulation and tumour suppression.

Selected Talk – S15

Structural heterogeneity of cellular K5/K14 filaments as revealed by cryo-electron microscopy

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Keratin intermediate filaments are an essential and major component of the cytoskeleton in epithelial cells. They form a stable yet dynamic filamentous network extending from the nucleus to the cell periphery. Keratin filaments provide cellular resistance to mechanical stresses, ensure cell and tissue integrity in addition to regulatory functions. Mutations in keratin genes are related to a variety of epithelial tissue diseases that mostly affect skin and hair. Despite their importance, the molecular structure of keratin filaments remains largely unknown.

In this study, we analyzed the structure of keratin 5/keratin 14 filaments within ghost keratinocytes by cryo-electron microscopy and cryo-electron tomography. By averaging a large number of keratin segments, we have gained insights into the helical architecture of the filaments. Interestingly, twodimensional classification revealed profound variations in the diameter of keratin filaments and their subunit organization. Reconstitution of filaments of substantial length from keratin segments uncovered a high degree of internal heterogeneity along single filaments, which can contain regions of helical symmetry, regions with less symmetry and regions with significant diameter fluctuations. Cross section views of filaments revealed that keratins form hollow cylinders consisting of multiple protofilaments, with an electron dense core located in the center of the filament.

These findings shed light on the complex architecture of keratin filaments, which demonstrate a remarkable degree of heterogeneity, suggesting that they are highly flexible, dynamic cytoskeletal structures.

Selected Talk – S16

Numerical Analysis of Keratin Networks in Selected Cell Types

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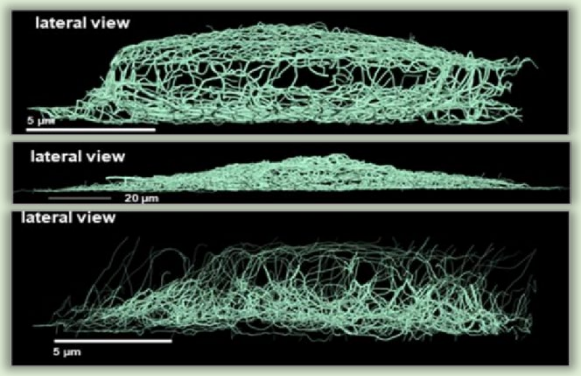
Keratin intermediate filaments make up the main intracellular cytoskeletal network of epithelia and provide, together with their associated desmosomomal cell-cell adhesions, mechanical resilience. Remarkable differences in keratin network topology have been noted in different epithelial cell types ranging from a well-defined subapical network in enterocytes to pancytoplasmic networks in keratinocytes. In addition, functional states and biophysical, biochemical and microbial stress have been shown to affect network organization. To gain insight into the importance of network topology for cellular function and resilience, quantification of 3D keratin network topology is needed.



Cinematic rendering of the keratin network in a n MDCK cell (top) , a human k eratinocyte (middle), and a native murine retinal pigment epithelium cell (bottom).

We used Airyscan superresolution microscopy to record image stacks with an x/y resolution of 120 nm and axial resolution of 350 nm in canine kidney-derived MDCK cells, human epidermal keratinocytes and murine retinal pigment epithelium (RPE) cells. Established segmentation algorithms (TSOAX) were implemented in combination with additional analysis tools to create a numerical representation of the keratin network topology in the different cell types. The resulting representation contains the xyz position of all filament segment vertices together with data on filament thickness and information on the connecting nodes. This allows the statistical analysis of network parameters such as length, density, orientation and mesh size. Furthermore, the network can be rendered in standard 3D software, which makes it accessible at hitherto unattained quality in 3D. Comparison of the three analyzed cell types reveals significant numerical differences in various parameters.

Abstracts – Flash Talks

Flash Talk F01

Impact of desmoglein 2 mutations on desmosomal integrity and stability

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Desmosomes are multiprotein complexes encompassing transmembrane adhesion proteins of the desmoglein and desmocollin type that are associated with linker proteins such as desmoplakin, plakoglobin and plakophilins, which together serve as anchorage sites for cytoplasmic intermediate filaments. Desmosomes provide mechanical tissue stability. Mutations in desmosomal genes therefore lead to diseases of mechanically challenged organs such as the skin and heart. Inherited arrhythmogenic cardiomyopathy (ACM) has even been labelled as a "disease of the desmosome".

The goal of the current study was to understand, how desmosomal protein mutations contribute to desmosomal function and dysfunction, especially with respect to ACM. We focused on desmoglein2 (Dsg2), which is the second most frequently affected gene in ACM patients. We prepared a series of human Dsg2 mutants to disrupt functionally- and disease-relevant properties. Wild-type and mutant human Dsg2 protein versions were introduced into epithelial MDCK cells, whose endogenous Dsg2 gene had been inactivated by CRISPR/Cas9 gene editing. Detailed analyses were performed using stably transfected cell lines.

We found that wild-type human Dsg2 functionally replaced the endogenous canine Dsg2 as determined by subcellular localization, electron microscopy, and dispase assays. Interestingly, all cell clones producing mutant Dsg2 versions showed reduced Dsg2 protein levels in comparison to human wildtype control cell clones whereas the Dsg2 mRNA levels were even enhanced. This suggested that the mutant Dsg2 polypeptides were less stable than the wild-type counterparts. Nonetheless, mutant Dsg2 polypeptides still localized at distinct punctate plasma membrane sites. Our observations further indicated that the equilibrium between desmosomal and extradesmosomal Dsg2 was shifted towards desmosomal integration in all mutants. The mechanical stability of some but not all MDCK cell clones harbouring human Dsg2 mutants was impaired. In addition, recent data suggest an impact of Dsg2 mutations on the overall keratin 8 network organization.

The newly developed cell lines provide promising platforms for investigating mechanisms how Dsg2 point mutations affect desmosome morphogenesis and stability. They will also help to understand, how Dsg2 mutations affect the organization of the associated cytoskeletal network and possibly other non-desmosomal pathways.

Flash Talk F02

GFAP splice variants differentially regulate glioma cellular dynamics and tumour growth

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Shared first authorship

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Gliomas are the most common form of malignant primary brain tumours in adults. The highly invasive nature of the tumour makes the disease incurable to date, emphasizing the importance of gaining a better understanding about the mechanisms of glioma invasion. Glial fibrillary acidic protein (GFAP) is an intermediate filament protein that is characteristic for astrocyte- and neural stem cell derived gliomas. Glioma malignancy is associated with changes in GFAP alternative splicing, as canonical isoform GFAPa is downregulated in higher grade tumours, leading to an increased dominance of GFAPd in the network. In this study, we investigate how this switch in the GFAPd/a ratio affects glioma cell behaviour using intravital imaging and an ex vivo brain slice invasion model. We show that the GFAPd and GFAPa isoforms differentially regulate the growth dynamics of gliomas. Depletion of either isoforms increases the invasive capacity of glioma cells. However, in GFAPd-KO cells this is mainly driven by increased motility, whereas GFAPa-KO cells show more persistent cell migration. This study shows that the composition of the GFAP network can drive specific migratory behaviours and affect the growth dynamics of glioma.

Flash Talk F03

VIMENTIN IN WILD TYPE AND MUTANT BONE PRECURSER-CELLS

Johanna Besold*, Claudia Hufnagel, Cindy Simon, Silvia Spitzer, Norbert Hassler, Jochen Zwerina and Thomas Dechat

Ludwig Boltzmann Institute of Osteology at Hanusch Hospital of OEGK and AUVA Trauma Centre Meidling, 1st Medical Department, Hanusch Hospital, Vienna, Austria; *presenting author

Despite its rigid structure, bone is a vital, dynamic organ which undergoes constant remodelling. While osteoclasts remove mature bone from the skeleton (bone resorption), osteoblasts are responsible for the formation of new bone (ossification). Osteoblasts derive from mesenchymal stem cells (MSCs) and after their maturation they build up the organic bone matrix and deposit mineral. Once bone formation is finished, osteoblasts either differentiate further into osteocytes, which become embedded into the bone, become so called bone lining cells or experience apoptosis. During these various differentiation steps the cell has to undergo various morphological changes. Since intermediate filaments (Ifs) play an essential role in determining cell morphology and shape we aim to investigate the role of vimentin during the differentiation of MSCs into osteocytes. As model cell lines we employ mouse mesenchymal stroma cells (D1 cells) and mouse pre-osteoblasts (MC3T3-E1 cells). Initially, vimentin expression and localization in these cell lines was investigated by immunoblotting and indirect immunofluorescence microscopy. Vimentin expression could be confirmed in both cell lines. Interestingly, its cellular staining pattern was highly dependent on the antibodies used. Using a polyclonal goat antibody against vimentin (“Traub”-antibody) gave a typical IF staining pattern with filaments reaching from the nucleus into the cytoplasm towards the nuclear periphery. However, using a commercially available mouse monoclonal anti-vimentin antibody gave a very different pattern, with a more “stress fibre like”-appearance in the case of MC3T3-E1 cells and a more punctuated staining throughout the cytoplasm with respect to D1 cells. This leads us to the speculation that different pools of vimentin exist within these cells whose detection is antibody dependent. Furthermore, we studied the cellular distribution of vimentin in the above mentioned cell lines lacking A-type lamins or kinesin-1, respectively. While lamin A/C deficiency led to less vimentin within the cytoplasmic region around the nucleus, knock-out of kinesin-1 resulted in the opposite, as vimentin became more concentrated around the nucleus. In addition, regarding vimentin structures stained with the monoclonal antibody, loss of the microtubule motor protein caused a less “stress fibre”-resembling pattern in the MC3T3-E1 cells. In future studies we aim to investigate vimentin distribution during osteoblast generation and differentiation in the wild type as well as in the mutant cell lines.

Flash Talk F04

Advanced cerebral organoid modelling of mutant GFAP

Werner Dykstra[1], ALEXANDER consortium, R. Jeroen Pasterkamp[1] & Elly M. Hol[1]

Department of Translational Neuroscience, University Medical Center Utrecht Brain Center, Utrecht University, Utrecht, The Netherlands

Alexander Disease (AxD) is a fatal neurological disorder caused by mutations in the astrocyte-specific glial fibrillary acidic protein (GFAP). The disease mainly affects children, is clinically characterized by loss of motor skills, seizures and cognitive decline and no cure exists. Neuropathologically, AxD is characterized by a loss of the fatty nerve conductor myelin and strong upregulation of GFAP that aggregates into astrocytic, cytoplasmic inclusions called Rosenthal Fibers (RFs). Astrocytes are a type of glial cells that serve numerous homeostatic functions in the brain. Upon central nervous system insult, astrocytes acquire a reactive state that can lead to a loss of homeostatic functions and to toxic gain of function. These "reactive astrocytes", a commonly observed phenomenon in many neurological diseases, are also characterized by an upregulation of intermediate filament proteins such as nestin, vimentin and GFAP. How mutations in the intermediate filament GFAP and subsequent astrocytic dysfunction lead to white and grey matter deterioration in AxD remains largely unknown. Animal models overexpresing (mutant) GFAP recapitulate certain AxD hallmarks such as RFs, but do not fully capture AxD pathology. Using AxD patient-derived induced pluripotent stem cells carrying the R239C mutation and a CRISPR/Cas9-corrected isogenic control line, we are studying neuron-glia interactions, as well as brain development in microglia-containing cerebral organoids. In this poster we will present our first data showing changes in AxD cerebral organoid development.

Flash Talk F05

The loss of keratin 19 increases susceptibility to experimental colitis but not to biliary injury.

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Medical Clinic III, Clinic for Gastroenterology and Metabolic Diseases, Aachen University Hospital, Aachen, Germany

Introduction: Keratins are the largest subgroup of intermediate filaments expressed primarily in epithelial cells. Simple epithelia produce a combination of type II (K7, K8) and type I keratins (K18/K19/K20/K23) are expressed in epithelia of the digestive organs. K8 and K18 are the only keratins found in adult hepatocytes, while cholangiocytes and intestinal epithelia express K7,K8,K18-K20.

Methods: We analyzed the biological importance of K7/K19 in digestive epithelia using constitutive knockouts lacking K7, K19 or both keratins (DKO). Colitis was induced by treatment with 2,5 % dextran sodium sulfate (DSS) in drinking water for 5 days. Biliary injury was mediated by crossbreeding the keratin KO mice with Mdr2KO animals.

Results: 3 months old K7/K19-KO and DKO mice developed normally and displayed histologically inconspicuous show biliary/intestinal systems, possibly due to compensatory altered K7, K8 and K18 expression. However, K19-KOs and DKOs had increased intestinal permeability to 4kD FITC-dextran. Seven days after DSS exposure, K19-KO/DKO but not K7-KO animals suffered significantly greater weight loss, shortening of the colon and greater epithelial inflammation. 16 months old, keratin-deficient Mdr2KO animals did not display significant alterations when compared to Mdr2KOs without keratin deficiency.

Conclusion: Our results demonstrated that K19 is essential for stress resilience of intestinal epithelia.

Flash Talk F06

Effects of specific phosphorylation sites on vimentin assembly and organization

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Cell Biology, Åbo Akademi University

Turku Bioscience Centre, Univ. of Turku and Åbo Akademi University

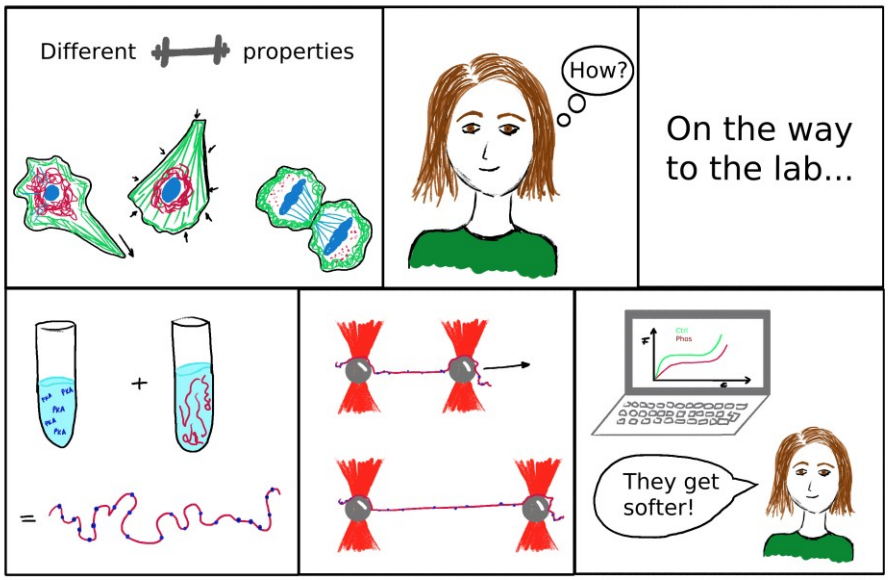
Vimentin is one of the most abundantly expressed intermediate filaments in cells of mesenchymal origin. It has been well-established that phosphorylation, a post-translational modification, is one of the most critical ways for cells to regulate vimentin dynamics, assembly, structure and function. It is still unclear which of the specific phosphorylation sites play the most crucial role in vimentin filament network assembly and organization. To address this question, we established phosphomimetic aspartate and phosphodeficient alanine point mutations at specific vimentin phosphorylation sites. This allowed us to analyze specific phosphorylated or dephosphorylated states in vimentin. Our results indicate that the serines 7, 8, 9 and serines 71, 72 of the vimentin Nterminal have the most prominent effect on the vimentin filament maturation, as the protein tends to form aggregates or globular structures following mutation. The other studied phosphorylation sites show only minor effects or no effect on the filament assembly. Our analysis also shows that phosphorylation of serines 4, 6 is not prominently involved in the filament maturation, but seem to be needed in regulating the soluble tetrameric pool of vimentin. Both a phosphomimetic and a phosphodeficient state of serines 4, 6 led to an increase in the amount of soluble, tetrameric vimentin. A similar effect was also seen with a phosphomimetic state of serines 7, 8, 9 and phosphodeficient state of serines 71, 72. Our results indicate that several phosphorylation sites of the vimentin N-terminal are critical in the regulation of protein assembly and solubility, where the phosphorylation state has to be tightly regulated for filaments to properly mature.

Flash Talk F07

Post-Translational Modifications Soften Vimentin Filaments

Julia Kraxner [1], Charlotta Lorenz [1], Sarah Köster [1]

[1] Institute for X-Ray Physics, University of Göttingen



Investigating the effect of phosphorylation on the mechanical properties of vimentin filaments.

Cells constantly need to adapt to various mechanical situations, for example during migration, contraction or cell division. A rather slow way to adapt cell mechanics to varying requirements on the cell is differential expression of the cytoskeletal proteins which affects the network architecture and the interaction between the filaments. Here, we focus on the intermediate filament vimentin and introduce post-translational modifications (PTMs), i.e. changes applied to specific amino acids in the protein after expression in the cell. By such PTMs, e.g. the charge pattern along the protein may be altered. Interestingly, PTMs occur comparatively fast and thus provide a mechanism for mechanical modulation on short time scales. We study the impact of one such PTM, phosphorylation, which is the addition of a phosphate group to an amino acid, on filament mechanics by stretching single filaments using optical traps. Whereas full phosphorylation leads to disassembly of IFs, partial phosphorylation results in softening of the filaments. By employing mutants that mimic phosphorylation, as well as Monte Carlo simulations, we explain our observation through the additional charges introduced during phosphorylation. This mechanism of fast adaptation of the mechanical properties of vimentin filaments could enable cells to fine-tune and adapt their mechanical properties according to changing requirements.

Flash Talk F08

Structural insight into disease-associated lamin mutations

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Abstract

The nuclear lamina is a meshwork consisting mainly of lamins and lamina-associated proteins, which is located in the inner nuclear membrane. It maintains the structural and mechanical stability of the nucleus, is involved in DNA repair and plays a major role in chromatin organization. Lamins are classified as type V intermediate filaments and in mammalian cells there are 4 different isoforms, A, C, B1 and B2. Mutations in human lamin genes lead to over 15 different diseases, called laminopathies, including muscle, metabolic and neuronal diseases, and can cause accelerated aging in the form of progeria syndrome. Almost all these diseases are linked to mutations in the *LMNA* gene, which encodes Lamin A and C. There are over 600 of these mutations known. However, to this date there is no fulllength high-resolution Lamin structure. To get one step closer to such a structure and to understand the disease-causing mutations we use cryo-electron tomography (cryo-ET), which has previously been capable of visualizing the nuclear lamina *in situ*. Additionally, quantitative fluorescence microscopy is applied to gain further insights into how the mutations affect both the nuclear lamina and the chromatin organization.

Until now, we have established and proven a workflow on the H222P mutation of the *LMNA* gene. This mutation is linked to Emery-Dreifuss muscular dystrophy and dilated cardiomyopathy. With great advances made in the image acquisition and data analysis we could push the resolution of the cryo-ET structural analysis further than before. We combined this with several light microscopy methods and showed how the mutation does not have a structural effect but rather how it leads to an increase in nuclear size and how it affects the chromatin organization. These results on the H222P mutation have now been published in the Journal of Cell Science and we focus our attention to a different laminopathy: Hutchinson-Gilford progeria syndrome (HGPS). By applying a similar workflow as with the H222P mutation, we hope to gain new insights into the structural effects of progeria-linked mutations on the nuclear lamina, the lamin filaments and the lamin-chromatin interaction, which is essential to understand this complex disease.

With the H222P mutation we also demonstrated a new approach to the sample preparation for cryoET, namely cryo-FIB milling. This approach allows us to visualize the nuclear lamina in its unaltered native environment. Thereby we have the potential to observe and analyse the interaction between lamins, chromatin and nuclear pore complexes. This is something which has not been possible before, due to the crowded environment at the nuclear envelope, small size of lamin filaments and the lack of resolution of other techniques. Thereby the combination of cryo-FIB and cryo-ET is the ultimate tool to finally visualize the nuclear lamina and the single lamin filaments inside the nucleus at high resolution.

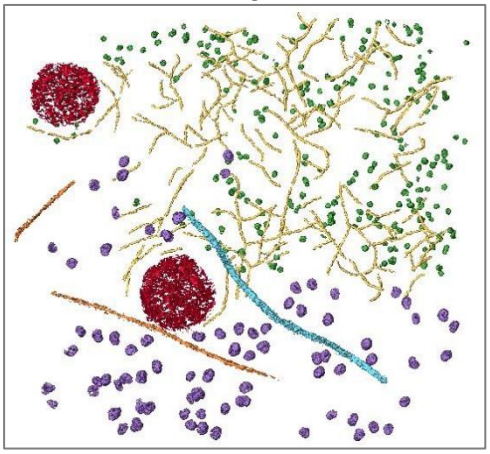

Figure: Segmented view of a cryo-EM tomogram of the nuclear envelope. Sample preparation by cryo-FIB milling. Lamin filaments (yellow), nucleosomes (green), NPC (red), ribosomes (purple), vimentin (blue), actin (orange).

Flash Talk F09

IF dimer plasticity as revealed by varied coiled-coil stability across the rod domain

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* Laboratory for Biocrystallography, KU Leuven, Leuven, Belgium

Despite a long history of structural studies of intermediate filaments (IFs), a thorough understanding of their molecular assembly process is still lacking. Multiple studies have proven the importance of dimer flexibility and local unravelling of the central coiled-coil region for filament assembly. The aim of our research is to experimentally assess the stability along the rod domain.

To this end, we have prepared six short (~50 residue) fragments of human vimentin which cover all three α-helical segments of the rod domain i.e. coil1A, coil1B and coil2. To facilitate the proper dimeric folding, the fragments were fused to N- or C-terminal capping domains. All constructs were shown to form dimers as confirmed by size exclusion chromatography coupled to multi-angle light scattering. Thereafter thermal melting experiments were performed using circular dichroism spectroscopy.

The N-terminal region of the central rod domain indicated a relatively low thermal stability that correlates with the previously proposed capacity of coil1A to unzip during assembly. Furthermore, the C-terminal fragment of coil2 displayed a modest melting temperature. In contrast, fragments including parts of coil1B as well as the C-terminal coil2 fragment showed higher thermal stability.

As a result, we were able to plot the stability landscape across the whole central rod domain of vimentin.

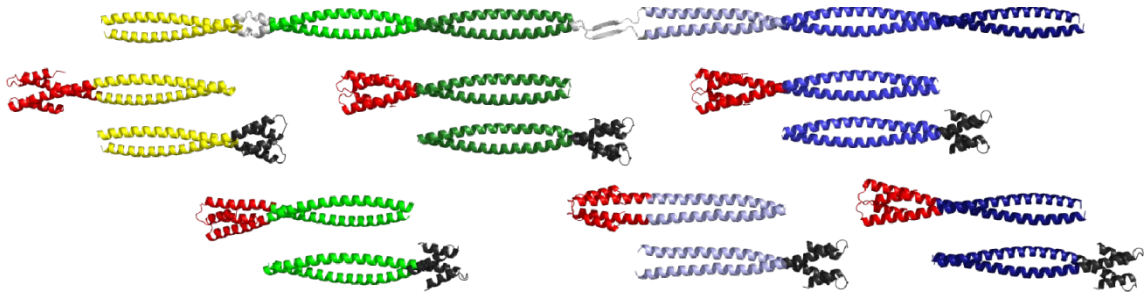


Figure. *Design of recombinant protein fragments for elucidating the thermal stability of the vimentin coiled coil.*

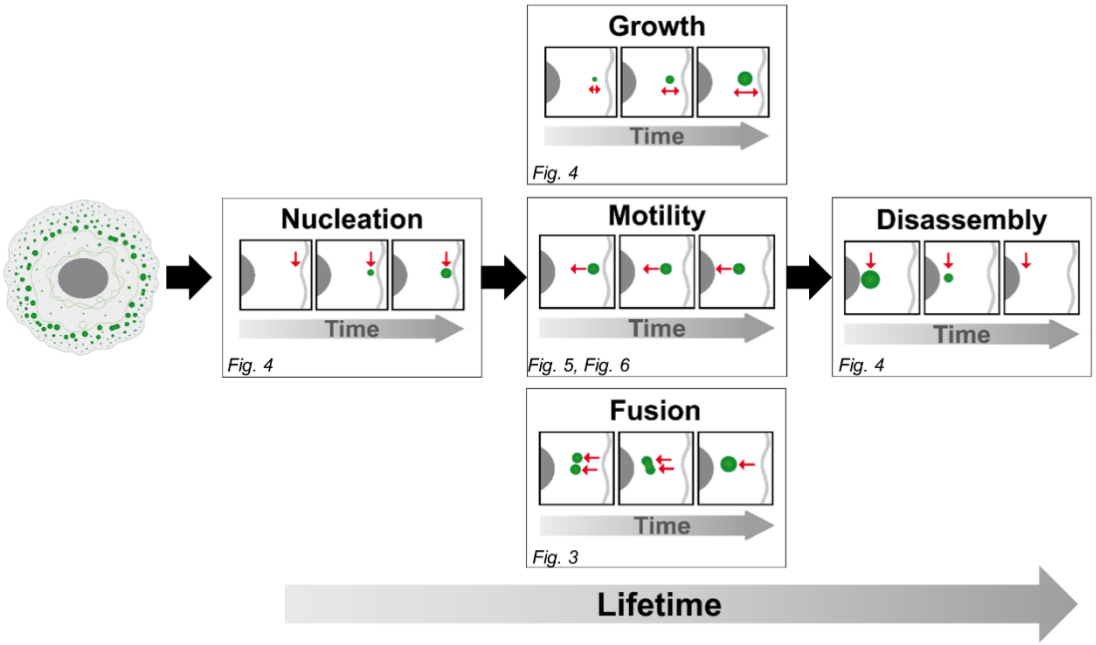
Acknowledgments: This research was performed in collaboration with Molecular Design and Synthesis group (KU Leuven) and Biochemistry, Molecular and Structural Biology group (KU Leuven).

Flash Talk F10

Tracking mutant keratin granules: Common features and myosin-dependent motility

Sonja M. Lehmann[1], Rudolf E. Leube[1], Reinhard Windoffer

[1] Institute of Molecular and Cellular Anatomy, RWTH Aachen University, Aachen



Intermediate filament polypeptides (IFPs) are prominent components of cytoplasmic aggregates that are pathognomonic for multiple diseases affecting various tissues. The previously held view that these protein aggregates are static terminal waste products, which accumulate over time, is being challenged by more recent observations, which suggest that they are dynamic and subject to regulated turnover. The emerging concept is that multiple factors contribute to the motility and turnover of IFP-containing aggregates. To understand their relative contribution, quantitative tools are needed. The current study addresses this need using epithelial cells producing mutant keratin IFPs that have been identified as the cause of the hereditary blister-forming skin disease *epidermolysis bullosa simplex*. These cells generate granular keratin aggregates that can be tracked individually by digital image analysis. This approach allowed mapping of the complete life cycle of single granules from assembly to disassembly, with information on position, protein concentration, size and motility at any given time-point. The deduced signet features revealed a limited granule lifetime of less than 30 minutes consisting of a slow but continuous growth phase followed by fast disassembly, rapid fusion of granules, and directed transport of growing granules from the cell periphery to the cell centre. As a paradigmatic proof-of-principle, we demonstrate that inhibition of non-muscle myosin II selectively reduces speed and directionality of granule movement linking keratin granule motility to the retrograde cortical acto-myosin flow. The newly developed tools and established parameters will help in the characterization of known and the identification of novel regulators of IFP-containing aggregates.

Flash Talk F11

Solving the puzzle: IF structure from dimer to the ULF

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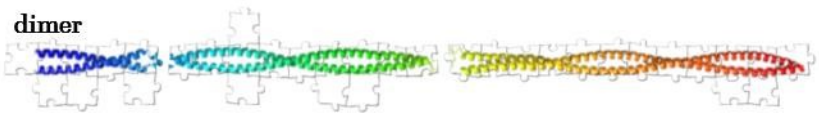
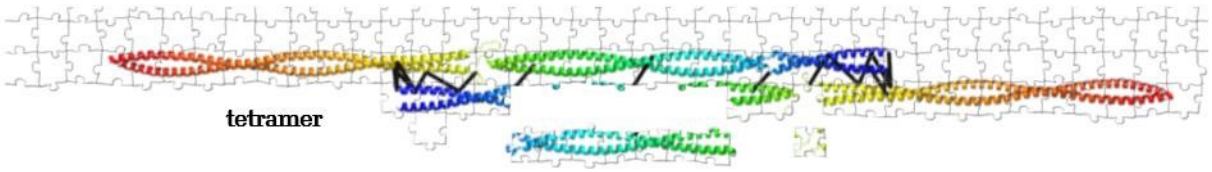
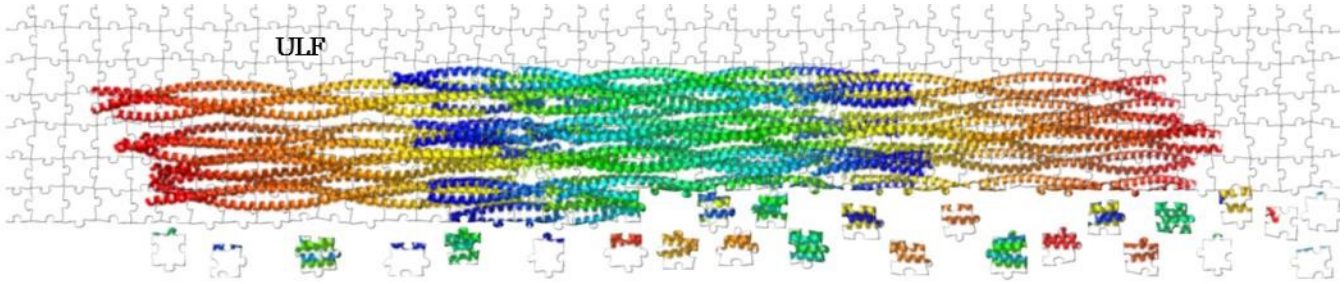
Vimentin is one of the most studied members of the intermediate filament family. Nevertheless, the complete structure of vimentin IFs is not available yet. The molecular mechanism of assembly and details of high assembly intermediates remain elusive. We investigate the molecular interactions that drive vimentin assembly by combining a range of structural biology techniques.



Atomic data on vimentin dimer provide a foundation for the research of higher assemblies. X-ray crystallography has been central towards obtaining detailed information on IF dimer and tetramer. Today we have the structural knowledge of the whole α-helical central rod domain of vimentin. The only missing parts are intrinsically disordered head and tail domains and linker regions.



However, X-ray crystallography could not resolve all dimer-dimer contacts that take place in the assembled filament. Instead, we use cross-linking coupled to mass-spectrometry to obtain essential distance restraints for computational docking of vimentin dimers.



Cryo-electron microscopy is highly promising for elucidating the structures of complex assemblies. Here we will discuss our progress on the high-resolution structure of vimentin ULFs.

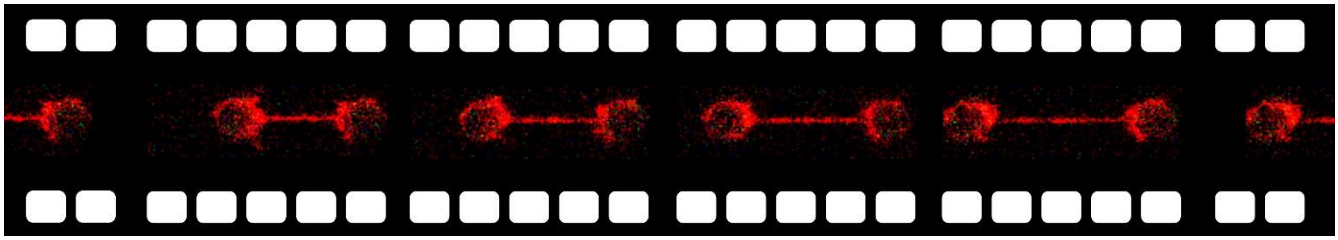
Integrative approach towards IFs can help to overcome their extreme flexibility and heterogeneity. With supporting information from different sources, we are getting closer to reaching atomic resolution.

Flash Talk F12

Keratin dimers slide, vimentin dimers don't – what are the consequences?

C. Lorenz and S. Köster

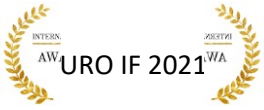
Institute for X-Ray Physics, University of Göttingen, Germany



Unlike actin filaments and microtubules, intermediate filaments (IFs) are expressed in a cell-type specific manner providing the cell with a tool to adapt to different mechanical requirements. Such adaptions play an important role, for example, for the endothelial-to-mesenchymal transition (EMT) during embryogenesis, wound healing and cancer metastasis. During EMT, the expression of vimentin and keratin varies, and, in parallel, the mechanical properties of the cell change. We thus hypothesize that differences in mechanical properties are already present on the single filament level and therefore study the physics of individual vimentin and keratin intermediate filaments, in terms of extensibility, energy dissipation upon repeated stretching, and softening.

We employ optical tweezers for our studies, which allow for precise measurements of force-strain relationships. We find that both filament types dissipate a large amount of the input mechanical energy, which predestines them to act as a cellular ``shock absorbers''. Yet, keratin filaments are softer than vimentin filaments so that they have to be stretched further to absorb the same energy. Whereas vimentin filaments do not plastically extend upon repeated stretching, keratin filaments permanently elongate to a certain extent, but maintain their stiffness. With help of a Monte-Carlo simulation we can qualitatively explain the differences in the behavior of keratin and vimentin filaments. Our simulation results support the hypothesis that keratin dimers can slide more easily with respect to each other than vimentin dimers. These different dimer sliding abilities may be explained by the different electrostatic, hydrophobic and compaction properties of keratin and vimentin. Our findings emphasize that cell mechanical properties can be traced by to the molecular length scale and that cellular and molecular biophysics are tightly connected.

COMING SOON



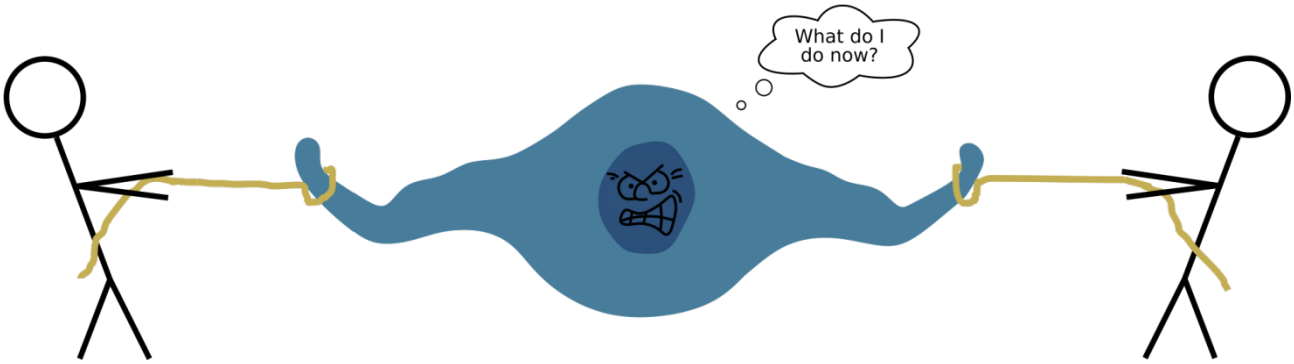
A KÖSTER GROUP PRODUCTION

Flash Talk F13

Cytoskeletal Networks in Cells Under Strain

R. Meyer, A. Schepers, P. Luley and S. Köster

Institute for X-Ray Physics, University of Göttingen



Investigating the function of the keratin cortex in the cell at high strains.

Cells in tissue permanently experience stress from the environment. For example, myocytes and alveolar epithelial cells are able to withstand deformations during muscle contractions and breathing movements, respectively. Intermediate filaments (IFs), which are expressed in a cell-type specific manner, allow cells to adjust to these various mechanical challenges. In some cells, the IF keratin forms a layer close to the membrane interconnecting the desmosomes in a circumferential network which may be referred to as an “IF-cortex”. It is hypothesized that this IF-cortex is part of a rimand-spokes arrangement of IFs in epithelia. Based on this hypothesis, IFs would add different mechanical properties to the cellular cortex than actin filaments do. Stretching experiments can help to study the behavior of networks and single filaments when experiencing stress coming from the environment. When comparing the results of experiments using microrheology to stretch the networks in vitro, the stress-strain behavior of the different cytoskeletal filaments vary significantly. While actin filaments and microtubules rupture at relatively low forces, IFs resist large forces and remain undamaged. Furthermore, when stretching single IFs, it was also shown that IFs display a plateau region, in which little force is needed to extend the filaments. Therefore, we are now interested in whether this stretching behavior of single IFs is also relevant in the filament network within a cell. The experiment is conducted by simulating the biological environment in tissue under controllable circumstances using an elastic surface. Here, a uniaxial cell stretcher is used to perform these experiments. Madin-Darby-Canine-Kidney cells (MDCK cells) are adhered to the elastic surface made from polydimethylsiloxane (PDMS). This device is then stretched uniaxially to high strains, overcoming the plateau regime of IFs. By comparing the stretching response of keratin knock-down cells to wild type cells in this experiment, we elucidate the role that the keratin layer plays at the cortex in the cells at high strains.

Flash Talk F14

Role of vimentin intermediate filaments in extracellular matrix organization and remodelling

Mayank Modi[1,2], Leila Rato[1,2], Peiru Yang[1,2], Guillaume Jacquemet[1,2], John E. Eriksson[1,2]

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Vimentin is a cytoskeletal protein having special properties which allow it to adapt under mechanical stress. Its stiffness increases exponentially under high strains and it undergoes structural changes. This enables cells to sense and respond to the mechanical stresses such as matrix stiffness and shear stress. Mechanical signals sensed by integrins on the cell surface are transduced to the cell through vimentin and other cytoskeletal proteins. This mechanosensing is essential for normal functioning of the body and facilitates the body to respond to physical trauma such as wounding. In wound healing, the tissue undergoes different processes such as homeostasis, inflammation, reepithelization, and extra-cellular matrix deposition and remodelling to regenerate damaged tissue. Mechanical stimulation of the wound through compression bandages aids in improved healing and decreases scarring. ECM remodelling is key to scar formation and this can be prevented by mechanical stimulation, however, the underlying process is unexplored. Vimentin in fibroblasts is implicated to be a key protein in wound healing and ECM remodelling due to its mechanosensing abilities.

Using cell-derived extracellular matrices from mouse embryonic fibroblasts as a model, we observed that Vim-/- MEFs show higher collagen type 1 production and lower fibronectin production. There seems to be an increase organization (fibrillogenesis) of exogenous fibronectin under the cell by Vim-/- MEFs, although the fibril thickness is similar to WT. The orientation of fibronectin fibrils shows lesser variation in Vim-/-, even though the overall alignment is similar to WT. Interestingly, there is an apparent increase in beta1 integrin activation and clustering in Vim/- MEF on collagen type 1 coated surfaces as evident from their smaller size and higher intensity. On stimulating remodelling, the ECM remodelled by Vim-/- MEFs shows lower alignment as compared to unremodelled ECM while WT MEFs maintain the apparent alignment going so far as to streamline the disarray in orientation by decreasing the variation in alignment. On examining further, there an increase in traction forces exerted by Vim-/- MEF on the substrate which could explain previously observed findings. These findings indicate that vimentin has a multifactorial role in ECM machinery which is crucial to several phenomenon such as wound healing, embryo development, and cancer metastasis.

Flash Talk F15

Vimentin protects cells from oxidative stress

Ponnuswamy Mohanasundaram[1, 2], Leila S Coelho Rato[1, 2], Peiru Yang[1, 2], Mayank Modi[1, 2], Fang Cheng[1, 2,3] and John E Eriksson[1, 2,]

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Reactive oxygen species (ROS) are highly reactive molecules, which can cause cellular damage and mutation. However, moderate amount of ROS regulates different cellular functions and signaling. ROS activate and maintain growth signals through inhibiting phosphatase activity. To maintain optimal redox level, cell generate antioxidant molecules and also switches it metabolism from aerobic to anaerobic glycolysis. In a highly proliferating cells, most glucose molecules are switch toward the pentose phosphate pathway, which supports macromolecules synthesis and NADPH production. NADPH maintain antioxidant glutathione (GSH) level by replenishing GSSG to GSH.

Vimentin is a type three intermediate filament protein and it abundantly express in cancer and mesenchymal cells. Vimentin regulates cancer cell invasion and proliferation, however, the molecular mechanism is not known yet. Here we found that stable expression of constitutively active KRasV12 in normal mammary epithelial cell MCF10A, changes its cell morphology from epithelial to mesenchymal and also increases vimentin level. Stable expression of KRasV12 increases cell migration quite drastically, however, vimentin knockdown reduces cell migration and increases oxidative stress. $Vim^{-/-}$ MEFs showed low level of GSH in steady state and mitochondria fragmentation after moderate amount of hydrogen peroxide treatment. Glucose uptake was also reduced in $Vim^{-/-}$ MEFs. Based on our observation, we hypothesis that vimentin could be important for maintaining growth signal and redox level in highly proliferative cells by modulating glucose uptake and metabolism. By using biochemical approaches, we are exploring the role of vimentin in oxidative stress protection.

Flash Talk F16

Vimentin from Adipocyte Progenitors Protects Human Dermal Fibroblasts against Osmotic Stress

Sepideh Parvanian[1,2,3], John Eriksson[1,2], Fang Cheng[1,2,3]

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[3]School of pharmaceutical science (Shenzhen), Sun Yat-sen University, Guangdong, Republic of China;

Introduction

We have previously showed that vimentin incorporated into the exosomes (exosomal vimentin) from pre-adipocytes is involved in wound healing by mediating fibroblast's activities such as proliferation, migration, and extracellular matrix accumulation. Cytoskeletal proteins including vimentin plays an important role in the cell resistance to mechanical stress and protection against apoptosis. Here, prompted by our previous findings underlying the crucial role of exosomal vimentin in promoting wound healing, we aimed to investigate its contribution to cell mechanical stress during wound healing and specifically osmotic stress condition.

Methods

Differential centrifugation was used for the isolation of EVs from wild-type (WT) and vimentin knockout 3T3lt1 (VIM-/-) cells. Electron microscopy and Western blot were used to characterize EVs from the cell culture media. In vitro analysis of cell migration, proliferation and collagen accumulation were performed using wound scratch assay, cell-derived matrices (CDM) and collagen staining in response to WT and VIM-/- exosomes.

Results

Our data shows that while osmotic stress increases the size and production of exosomes, WT exosome promotes activities of osmotic- stressed fibroblasts and protect cells against apoptosis.

Furthermore, our findings indicated that exosomes may act as a complex information package to either restore the osmotic balance or to induce osmotic stress-driven condition in normal cells, while exosomal vimentin significantly contributed to this process.

Summary/Conclusion

This study is expected to reveal a novel role for exosomal vimentin in regulating fibroblast activities in osmotic stress conditions during wound healing.

Funding

This project funded by Sigrid Juselius Stiftelse and Åbo Akademi University.

Key words: Exosomal vimentin, Adipose-derived Stem Cells, osmotic stress, Wound healing

Flash Talk F17

Extracellular Matrix Heterogeneity and Retinal Mechanobiology: Does the keratin network control mesoscale properties of the pigment epithelium?

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Epithelial cells are highly interconnected, creating a cellular continuum able to accomplish specific tissue functions. This is particularly challenging in postmitotic epithelia, where the natural cell loss is compensated by dynamic cellular reconfiguration to maintain tissue integrity. One important example is the retinal pigment epithelium that, owing to the ability to tolerate monolayer defects, ensures the functionality of the retina in ageing with cellular deformation and multinucleation. The retina detects light via photoreceptor cells, which homeostasis depends on the direct contact with the retinal epithelium monolayer. Interestingly, the monolayer organization and its cytoskeletal elements have been suggested to regulate the ability to support photoreceptor cells homeostasis.

Like in every epithelium, the extracellular matrix regulates tissue function by its molecular composition and physical cues. This is due to emerging mesoscale properties that cannot be predicted by studying single cells and depend on the equilibrium of epithelial intercellular tension and the adhesion forces on the extracellular matrix. The combination of retinal epithelium postmitotic nature and age-related extracellular matrix remodelling open the unexplored question of how monolayer organization and mechanical forces regulate retinal function during physiological and pathological ageing.

To address this question, with our work we aim to characterize the relation between retinal epithelium mechanics and its functionality using a stem cell-derived model *in-vitro* with the reference of the *in-vivo* retina. Our data show that molecular rather than physical cues stemming from the extracellular matrix play a pivotal role in controlling epithelial monolayer mechanics. In particular, the *in-vivo* heterogeneous distribution of the basement membrane laminin α3 corresponds to retinal epithelium intercellular stress distribution, monolayer arrangement and keratin 8 network organization. These epithelial features can be phenocopied *invitro* using an inert hydrogel system coated with different densities of laminin α3. Current work focuses on understanding the specific role of the three-dimensional Keratin 8 network in controlling retinal epithelium mechanics in relation to the density of laminin α3-binding hemidesmosomes and cellular ability to internalize photoreceptor outer segments.

Flash Talk F18

Autophagy and endoplasmic reticulum stress contribute to the pathogenesis of arrhythmogenic cardiomyopathy in desmoglein 2-mutant mice

Mark Pitsch – Sebastian Kant – Rudolf E. Leube – Claudia A. Krusche

Institute of Molecular and Cellular Anatomy, RWTH Aachen University, 52074 Aachen, Germany

Arrhythmogenic cardiomyopathy (AC) is caused by mutations of desmosomal genes, including the desmosomal cadherin desmoglein 2 (Dsg2). In murine AC models structural disease onset is characterized by focal cardiomyocyte necrosis, which elicits a multiphase inflammatory response and subsequent replacement fibrosis. Further disease progression is driven by cardiomyocyte hypertrophy, interstitial fibrosis and chronic inflammation. Based on the altered expression of genes regulating calcium-handling in AC-mutant mice and the postulated enhancement of autophagy in various cardiomyopathies, we pursued the hypothesis that endoplasmic reticulum (ER) stress and autophagy contribute to AC disease progression.

To this end, we examined myocardial samples derived from two Dsg2-mutant mouse lines during disease initiation and progression (4 to 50 weeks). We assessed expression of established markers of the unfolded protein response (UPR) and autophagy by qRT-PCR (p62, CHOP, sXBP1, uXBP1), immunohistology (LC3B, p62) and in situ hybridization (CHOP, p62).

Increased expression of the autophagy markers p62, CHOP and LC3B was detected at the start of replacement fibrosis formation in 4 week-old Dsg 2-mutant hearts. Significantly elevated mRNA levels of the UPR markers uXBP1 and sXPP1 were observed at the same time suggesting that ER stress triggers autophagic activity. Strikingly, LC3B an p62 protein expression as well as CHOP mRNA expression was restricted to cardiomyocytes next to the forming replacement scars. LC3B and p62 protein expression was also detectable later during disease progression (up to 50 weeks) predominantly in cardiomyocytes, that were localized in the vicinity of established fibrotic scars. To find out, whether calcium handling deficits may contribute to the enhancement of autophagic activity during advanced disease stages, mRNA expression of the Na^{+}/Ca^{2+} exchanger NCX1 and the cardiac ryanodine receptor calcium release channel RyR2 were examined. NCX1 and RyR2 mRNA expression was impaired especially in the more heavily affected right ventricles.

In summary, our observations support the hypothesis that enhanced autophagy plays an important role at the beginning and during chronic progression of AC pathogenesis. The data further indicate that the UPR of the ER is a major trigger of autophagy during the early disease phase and that impaired calcium handling contributes to excessive induction of autophagy during late AC stages. Both inductive responses are potential new drug targets to modulate AC disease progression.

Flash Talk F19

Epiplakin-keratin interactions revisited – towards unraveling the biological functions of a mysterious giant protein.

Sonia Ratajczyk[1]*, Corinne Drexler[1]*, Gerhard Wiche[1], Pavel Strnad[2], Rudolf Leube[3], Peter Fuchs[1]

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Epiplakin is a member of the plakin protein family and is exclusively expressed in epithelial tissues where it binds to keratins. Epiplakin-deficient mice show no discernible phenotype, except for a slight acceleration of keratinocyte migration. We could show that in several pancreas and liver stress models, epiplakin and keratin expression was upregulated in parallel. In addition, epiplakin-deficient mice suffered from aggravated injuries and their diseased organs contained larger amounts of keratin aggregates. Our data provide strong evidence that epiplakin is a protective protein in stress situations when keratin expression is upregulated, probably by acting as a keratin organizer preventing formation of harmful keratin aggregates. To elucidate these proposed functions we are using bio-optical and biochemical approaches to study epiplakin-keratin interactions in more detail. Live cell imaging using cultured cells expressing tagged keratins and epiplakin revealed an unexpected and yet undemonstrated diffuse cytoplasmatic localization of epiplakin during homeostatic conditions. This state quickly changed upon induction of several kinds of stress leading to perfect colocalization of epiplakin with keratin filaments. This characteristic of epiplakin was previously overlooked due to its almost complete keratin association taking place during fixation procedures preceding immunostainings of cells and tissues. Additional experiments are needed to clarify the molecular alterations leading to this quick change in epiplakin's binding affinities for keratins and to reveal the consequences of this interaction for keratin filament organization and dynamics in stressed cells and tissues.

Flash Talk F20

Development of a three-dimensional skin model to investigate the mechanical properties of keratin intermediate filaments

Jana Schieren[1], Ramin Nasehi[2], Horst Fischer[2], Nicole Schwarz[1], Rudolf E. Leube[1]

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Keratin intermediate filament networks play an important role in the organization and stability of skin keratinocytes by resisting forces acting on the epidermis. Mutations in keratin-coding genes lead to an impairment of the mechanical properties of the epidermis and consequently result in rare keratinopathies, which, depending on the affected keratins, show different phenotypes.

Research efforts to elucidate the mechanical properties of the different keratins and the consequences of their mutations have mainly focussed on experimental set-ups involving single cells or differentiating monolayers. In our work, we are therefore implementing skin models with an epidermal compartment grown from immortalized cell lines of healthy donors as well as from patient cell lines. We use *epidermolysis bullosa simplex* (EBS) and *pachyonychia congenita* (PC) as model diseases, because they involve different keratins and show clearly distinguishable phenotypes. Since these skin pathologies typically manifest in response to mechanical insults, we developed a device (Figure 1) to apply compressive load to the skin models in a controlled manner allowing to examine the consequences of defined mechanical stress.

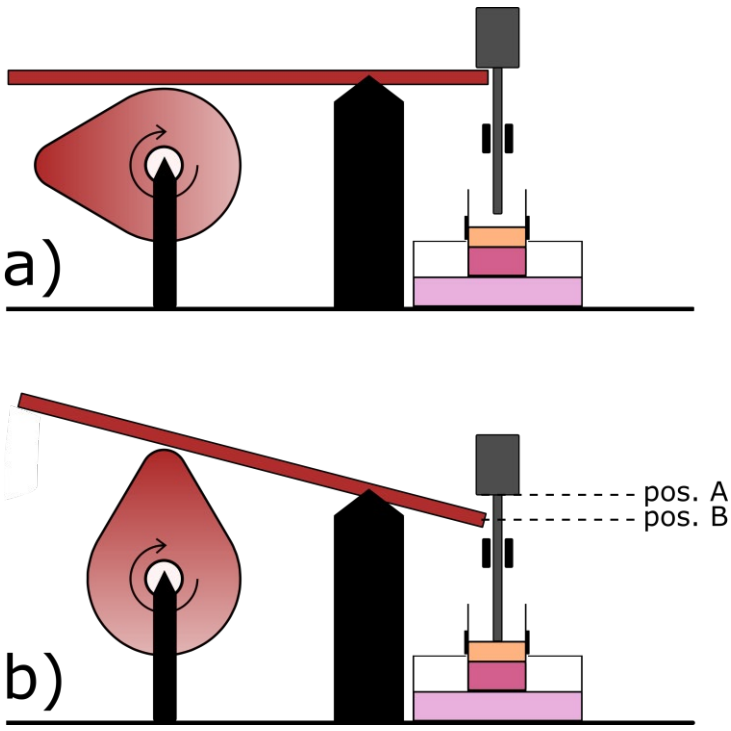

Figure 1: Schematic representation of a device for controlled compression of skin models.

An indenter with a flat, circular tip is moved up (a) and down (b) by a cam and follower mechanism driven by a DC motor. It can be lowered to maximum displacement (pos. B) or stopped by the surface of the sample (pos. A). In this way the thickness of the sample does not influence the procedure. A guide cone ensures vertical movement. The compression force and frequency are modulated by the weight of the indenter and the motor speed, respectively.

Flash Talk F21

Desmoplakin maintains transcellular keratin scaffolding and protects from intestinal injury

Annika Schmitz (Gross)[1], Biaohuan Zhou[1], Nicole Schwarz[2], Gabriel M. Schacht[2], Peter Boor[3], Konrad Hoeft[4], Bernd Hoffmann[5], Nurdan Güldiken[1], Elaine Fuchs[6], Rafael Kramann[4], Rudolf Merkel[5], Rudolf E. Leube[2], Pavel Strnad[1]

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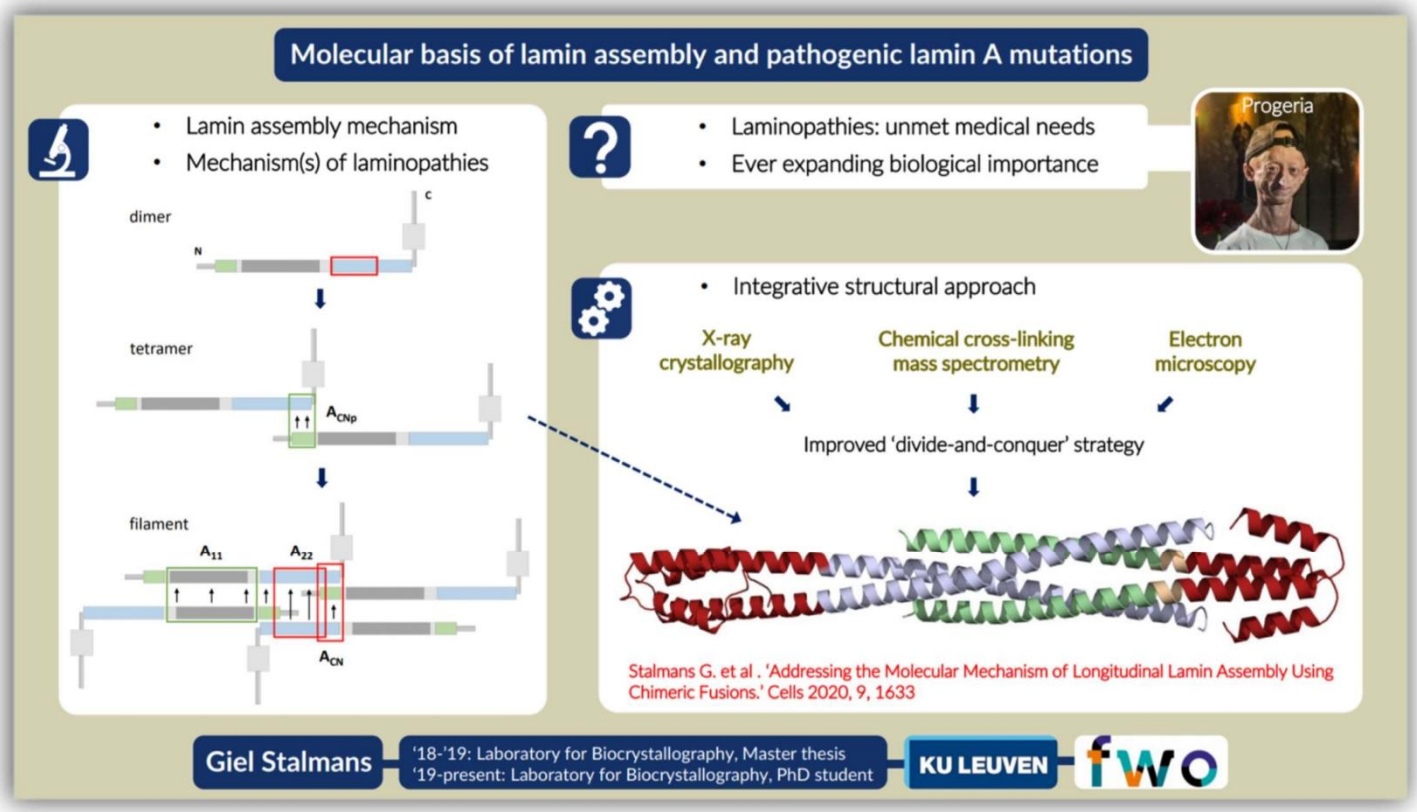
Background: Desmosomes are intercellular junctions connecting keratin intermediate filaments of neighboring epithelial cells. The cadherins desmoglein 2 (Dsg2) and desmocollin 2 mediate cell-cell adhesion, whereas desmoplakin (Dsp) provides the attachment of desmosomes to keratins. While the importance of the desmosome-keratin-network is well established in mechanically challenged tissues, we assessed its currently largely unknown function in intestinal epithelia.

Methods: We analyzed the intestinal phenotype of intestine-specific villin-Cre DSP ($DSP^{\Delta IEC}$) and combined, intestine-specific DSG2/$DSP^{\Delta IEC}$ (ΔDsg2/Dsp) knockout mice by histology, RT-PCR and immunoblotting. Cell proliferation was quantified after 5-bromo-2'-deoxyuridine (BrdU) application. Colitis was induced by treating the mice with 2.4% dextran sodium sulfate (DSS). Cross-breeding with keratin 8 (K8)-YFP knock-in mice and generation of organoids was performed to visualize the keratin network. A Dsp-deficient colorectal carcinoma HT29-derived cell line was generated via CRISPR/Cas and the role of Dsp in adhesion and mechanical stress was studied in dispase assays and after exposure to uniaxial cell stretching.

Results: The intestine of $DSP^{\Delta IEC}$ mice was histopathologically inconspicuous, but intestinal epithelial cells displayed an accelerated migration along the crypt and an enhanced shedding into the intestinal lumen. Increased intestinal permeability and altered levels of desmosomal proteins were detected. An inconspicuous basal phenotype was also seen in ΔDsg2/Dsp mice. After DSS treatment, $DSP^{\Delta IEC}$ mice suffered from a more pronounced colitis. A retracted keratin network could be delineated in the intestinal epithelium of $DSP^{\Delta IEC}$/K8-YFP mice and organoids derived from these mice presented a collapsed keratin network. In contrast, no differences in the expression, phosphorylation or solubility of keratins were observed. Dsp-deficient HT29 cells had an impaired cell adhesion and showed an increased cellular damage after stretch.

Conclusions: Our results demonstrate that Dsp is required for proper keratin network architecture in intestinal epithelia, mechanical resilience and adhesion thereby protecting from injury.

Flash Talk F22



Giel Stalmans[1], Anastasia V. Lilina[1], Pieter-Jan Vermeire[1], Jan Fiala[2,3], Petr Novak[2,3], Sergei V. Strelkov[1]

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Despite the upmost biological importance of nuclear lamina it is still not known how lamin filaments are formed at molecular level. The particular dimer-dimer interactions responsible for the assembly process are also poorly understood. This situation strongly limits any rational efforts to develop therapeutic approaches to laminopathies. We will discuss some recent results on lamin A structure and assembly following an improved 'divide-and-conquer' strategy. In particular, we utilize chimeric fusions made between the relevant parts of the lamin dimer and terminal capping motifs that stabilize the coiled coil. Such constructs and higher specific complexes thereof are being studied using X-ray crystallography and chemical cross-linking.

Flash Talk F23

Elucidation of 3D structure of vimentin tetramer through cryoelectron microscopy

Giulia Sultana, Peiru Yang, Emilia Holm, John E. Eriksson

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The vimentin network plays a key role in cell architecture and signaling. At structural level vimentin possess three domains: head, rod and tails. Vimentin has 23 bonafide phosphorylation sites on its N-terminus and 11 on C-terminus. In particular, certain phosphosites are specifically phosphorylated by one or two kinases.

Vimentin dynamics is regulated by phosphorylation. Site-specific phosphorylation induces disassembly of vimentin filaments, and the increase of phosphorylation supports vimentin filament reorganization in vivo. Vimentin is phosphorylated by different protein kinases at several sites. Although, several vimentin segments and partial oligomers have been crystallized, a crystal structure for full-length monomeric or tetrameric vimentin is not yet available. Molecular modelling studies propose that vimentin monomers assemble into parallel dimers that in turn associate in an antiparallel, staggered manner into tetramers, which are considered as the structural units for vimentin polymerization. Crosslinking studies have shown that several modes of assembly are possible for vimentin tetramers. Eight tetramers would assemble into ‘unitlength filaments’ that connect head to tail and compact to give the 10-nm-wide mature filaments.

Here, we induced vimentin expression in *E. coli* and purified the protein. We obtained a tetramer of vimentin. We plan study the tetramer by different massspectrometry-based methods following cross-linking and also to do cryoelectron microscopy on the tetrameric samples.

Flash Talk F24

Vimentin supports directional cell migration via focal adhesion modulation

Arun P Venu, Mayank Modi, Ujjwal Aryal, Elena Tcarenkova, Yaming Jiu, Guillaume Jacquemet, Johanna Ivaska, Alexander Minin, Fang Cheng, and John E Eriksson

Abstract

Fibroblastic migration is of key physiological importance in wound healing, in macrophagemediated immune responses, and in cancer metastasis. The intermediate filament protein vimentin is important for both wound healing and for normal fibroblast migration in healing wounds. However, the mechanisms underlying these effects are still poorly understood. Here, we show that vimentin affects directionality by guiding focal adhesions in fibroblasts during cell migration. In wound healing assays inducing cell polarization and directed migration, we show that vimentin-deficient mouse and rat embryonic fibroblasts completely lack directional persistence. Detailed analysis showed that vimentin stabilizes focal adhesions and regulates their disassembly rate. The destabilization of Vim-/- focal adhesions was reflected by smaller focal adhesions and lower amounts of paxillin, vinculin, and reduced autophosphorylation of focal adhesion kinase. Live cell imaging demonstrates that vimentin interacts dynamically with the key molecules of focal adhesions and, importantly, with focal adhesion kinase, which is crucial for the maturation of focal adhesions. Global orientation of focal adhesions during migration were also significantly affected in Vim-/- MEF cells. These results demonstrate that vimentin intermediate filaments are in dynamic bidirectional interplay with focal adhesion proteins, thereby controlling the maturation, stability, dynamics, arrangement, and overall orientation of focal adhesions, with a net effect on focal adhesion coordination during directional migration.

Flash Talk F25

Mechanical crosstalk between hemidesmosome, focal adhesion and integrin-containing clathrin lattices

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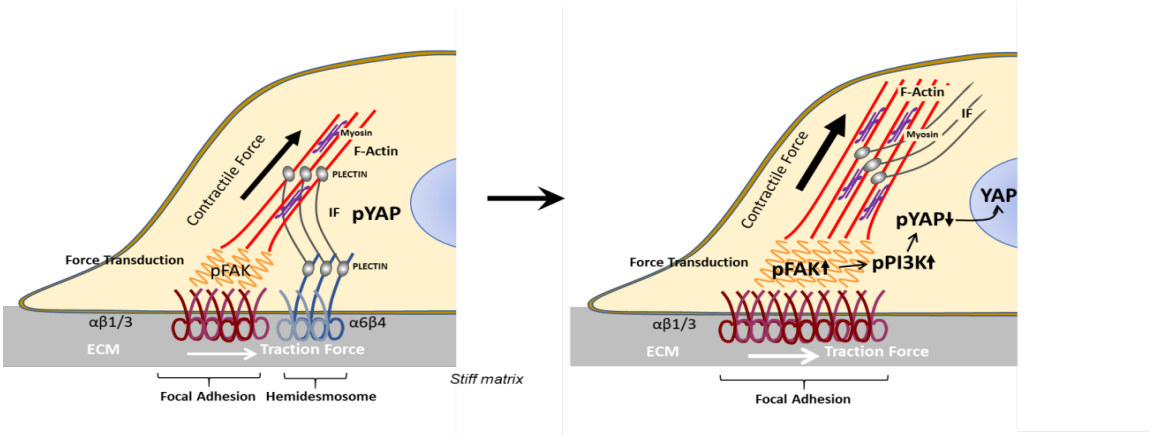
Integrin α6β4 is essential for the formation of hemidesmosomes, cell adhesion structures that stably anchor epithelial cells to the basement membrane by binding extracellular laminin and associating with plectin and the keratin intermediate filaments intracellularly. Although the adhesion function of hemidesmosomes has been extensively studied, their role in mechanosignaling and transduction remains largely unexplored. Here, we confirm that the intact laminin-integrin β4-plectin linkage is essential for maintaining HD structure and demonstrate that if this linkage is impaired, cell area, FA size, and actomyosin contractility are increased. Moreover, we measured traction forces on different substrates in the PA-JEB,PA-JEB/β4, and β4 mutant cell lines. The data showed that keratinocytes that cannot form intact HDs, generate higher traction forces, especially on stiffer matrices. We further demonstrate that integrin α6β4 regulates the activity of the mechanosensitive transcriptional regulator YAP through inhibition of Rho–ROCK–MLC– and FAK–PI3K–dependent signaling pathways. Additionally, increased tension caused by impaired hemidesmosome assembly leads to a redistribution of integrin αVβ5 from clathrin lattices to focal adhesions. Our results reveal a novel role for hemidesmosomes as regulators of cellular mechanical forces and establish the existence of a mechanical coupling between adhesion complexes.



Flash Talk F26

Hetero- and homophilic interaction of desmosomal cadherins

Sophia Wüllenweber[1], Rudolf E. Leube[1], Sebastian Kant[1]

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Desmosomal adhesion is mediated by two classes of desmosomal cadherins, i.e. desmogleins and desmocollins. An ongoing and hotly debated conundrum is, whether they interact hetero- or homophilically in physiological *in vivo* situations. This lack of knowledge even applies to simple epithelia, which express only one member of each multigene family, namely desmoglein 2 and desmocollin 2. Yet, knock-out mouse models have shown that formation of desmosomes or at least desmosome-like junctions occurs in simple epithelia even in the absence of either desmoglein 2 or desmocollin 2. It may still be true, however, that heterophilic interactions are the favored type of interaction as some *in vitro* studies suggest.

We therefore set out to systematically study desmosomal cadherin interaction in cultured cells using the previously described human colon carcinoma-derived DLD-1 subclone that had been depleted of desmoglein 2 and desmocollin 2 by targeted gene editing (Fujiwara et al., 2015, J Biochem 158:339). These desmosomal cadherin-deficient cells were transfected with expression constructs coding for desmoglein 2 or desmocollin 2 containing different fluorescence tags. The isolated cell clones were then mixed to examine homo- versus heterophilic interactions in trans by fluorescence microscopy. Clusters with homo- and heterophilic desmosomal cadherin pairs were detected. Heterophilic interactions, however, appeared to be preferred. Dispase assays and traction force microscopy helped to untangle differences in adhesion and force development of the different homo- and heterophilic desmosomal cadherin pairs.

Taken together, the newly described cell lines provide useful tools to analyze desmosomal cadherin pairing in quantitative terms in a vital context revealing different features of each cadherin type.

Flash Talk F27

The role of vimentin in lipid droplet development and lipid metabolism

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Lipid droplets (LDs) are recognized as organelles in a dynamic change to regulate lipid and energy homeostasis. The intermediate filament (IF) protein vimentin has been implicated to be involved in lipid metabolism. We observed that vimentin-deficient mice have demonstrated significant fat loss and complete resistance to high-fat diet. $Vim^{-/-}$ mice are leaner with a markedly lower bodymass index and fat/bodyweight ratio. Histological examination revealed that the adipocytes were significant smaller in different types of adipose tissue in the $Vim^{-/-}$ mice. Cage-like vimentin IF (vIFs) structure around LDs in mature adipocytes suggests an involvement of vimentin in lipogenesis. To investigate the role of vimentin in the context of lipid signaling and metabolism, we generated 3T3-L1 preadipocytes with vimentin deletion ($Vim^{-/-}$). Differentiated adipocytes were used as a model to examine the mechanisms underlying the lipodystrophy.

Similar phenotypes were observed in 3T3-L1 adipocyte. Confocal imaging detected that $Vim^{-/-}$ adipocytes develop much smaller and with a more uniformly small size distribution of the LDs, while LDs can grow much bigger with the presence of vIFs and also show much heterogeneity. Using RNA sequencing as an approach to study the transcriptional changes, RNAseq data demonstrated significant differences in late differentiation markers between the wild-type and $Vim^{-/-}$ adipocytes, indicating that the differentiation of the adipocytes was inhibited. Western blot demonstrated a higher level of lipophagy in $Vim^{-/-}$ adipocytes. We also observed that that inhibition of the mTORC1 signaling pathway, which indicates that mTORC1 signaling pathway is likely to be involved in the lipodystrophy phenotype of the vimentin-deficient adipocytes.

Flash Talk F28

Myonuclear number decreases during disease progression in *Lmna* mutant mice, and is not the result of impaired nuclear accretion

Hind Zahr*[1], Alexandra Corbin*[1], Alexa Podolsky[1], Liza Koper[2], Jan Lammerding[1], Tyler Kirby[2]

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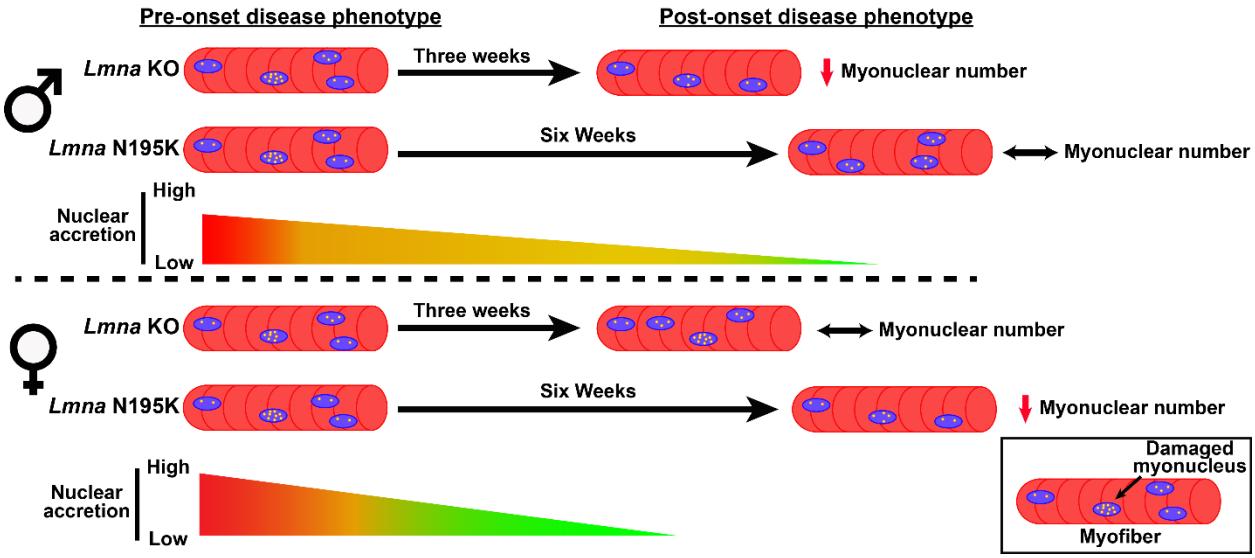
* contributed equally

Mutations in the nuclear envelope proteins lamin A and C cause various muscular dystrophies and dilated cardiomyopathy, as well as a variety of other diseases collectively referred to as laminopathies. The molecular mechanisms for the often muscle-specific defects remain unclear. We have previously shown that certain *Lmna* mutations affect nuclear stability in skeletal muscle cells. This loss of nuclear stability leads to nuclear envelope rupture, widespread DNA damage, and subsequent activation of DNA damage response pathways. Currently, the consequences of this nuclear damage in skeletal muscle fibers from *Lmna* mutant mice is unknown. Previous reports indicate that muscle fibers from lamin A/C-deficient mice (*Lmna*KO) have less myonuclei, leading us to hypothesize that perhaps the extensive nuclear damage was leading to myonuclear loss. To test this hypothesis, we examined myonuclear content in *Lmna* KO mice and mice homozygous for the *Lmna* N195K mutation *(Lmna* N195K) at two separate timepoints, corresponding to pre-onset and post-onset disease phenotype. In addition, to gain temporal information on myonuclear dynamics, we tracked the fusion of newly acquired myonuclei from muscle stem cells using EdU incorporation. We found that nuclear damage was associated with decreased myonuclear number in a sex-specific manner; following disease progression, decreased nuclear content was observed only in the male *Lmna* KO and female *Lmna*N195K mice. Importantly, this decrease in nuclear content was not the result of impaired stem cell function, as nuclear accretion was similar or greater in the*Lmna* mutant mice relative to *Lmna* WT controls. Intriguingly, we observed temporal differences in nuclear accretion in male and female mice, which were consistent with the sex-specific differences in myonuclear content between the *Lmna* KO and *Lmna*N195K strains. Collectively, our findings suggest that the lower myonuclear content observed in *Lmna* mutant mice is due to myonuclear loss, rather than defects in nuclear accretion. To provide insights into the mechanism underlying this myonuclear loss, we generated *Lmna* mutant strains lacking *Trp53* (p53). Preliminary results indicate that the myonuclear loss still occurs in *Lmna* mutant mice lacking p53, suggesting that myonuclei are being removed via a p53-independent mechanism. Future studies will aim to determine the mechanism by which myonuclei can be selectively removed from the muscle fiber syncytium in *Lmna* mutant mice.



Extensive myonuclear damage is associated with myonuclear loss in skeletal muscle cells from *Lmna* mutant mice.

Flash Talk F29

Epithelial actomyosin state determines how adherens junctions promote desmosome assembly

Hanyin Zhang[1,2], Barbara Boggetti[2,4], Plácido Pereira [1,2], Matthias Rübsam[1,2], Martijn Gloerich[3], and Carien M. Niessen[1,2,5]

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Abstract

Epithelia form physical and functional barriers that are exposed to a wide range of mechanical stresses. Cadherin-based adherens junctions (AJs) and desmosomes connect intercellular adhesion to actin and intermediate filament systems respectively, thus allowing neighboring cells to act as mechanical units to withstand and respond to these stresses. The classical cadherin-catenin complex forms the adhesive backbone of AJs and its engagement is essential for desmosome assembly. We previously identified an essential role for E-cadherin-Desmoglein-2 interactions in facilitating desmosome assembly. Using structure-function analysis in combination with immunofluorescence and ultrastructural analysis, we now explore the role of cadherin-complex-actin interactions in desmosome formation in simple (MDCK) and stratified epithelia (keratinocytes). Our data identify a central role for acatenin in desmosome assembly, but, surprisingly, while its actin-binding domain is sufficient in MDCK cells, desmosome stabilization required the force-dependent unfolding of its vinculin binding domain in primary keratinocytes. Atomic Force Microscopy revealed that keratinocytes are stiffer than MDCK cells and that upon induction of intercellular tension increased in keratinocytes but not MDCK cells. This increase was not seen in a-catenin-depleted keratinocytes. Importantly, increasing tension in MDCK or decreasing tension in keratinocytes would reverse a-catenin domain requirements for desmosome assembly. Together, our data suggest that next to initial recruitment of desmosomal cadherins to intercellular contacts via extracellular interactions with AJ cadherins, the cadherin-catenin complex also senses and counteract actomyosin contractility necessary for stabilizing desmosomes.

Attendees

Giulio Agnetti
John Hopkins University

Joana Ahlburg
RWTH Aachen University

Johanna Besold
Ludwig Boltzmann Institute of Osteology

Pascale Bomont
Inserm

Leila Sofia Coelho Rato
Abo Akademi University

James Peter Conboy
TU Delft

Thomas Dechat
Ludwig Boltzmann Institute of Osteology

Jacopo Di Russo
RWTH Aachen University

Werner Dykstra
University Medical Center Utrecht

Peter Fuchs
University of Vienna

Florian Geisler
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Emilia Holm
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Irene Isturiz
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Paul A. Janmey
University of Pennsylvania

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Jessy van Asperen
Utrecht University

Emma J van Bodegraven
Institut Pasteur

Arun P Venu
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Wei Wang
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Miriam Sarah Weber
University of Zurich

Sophia Wüllenweber
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