33rd Ernst Klenk Symposium in Molecular Medicine

Tissue regeneration, wound healing and fibrosis: Translating basic concepts into regenerative therapy

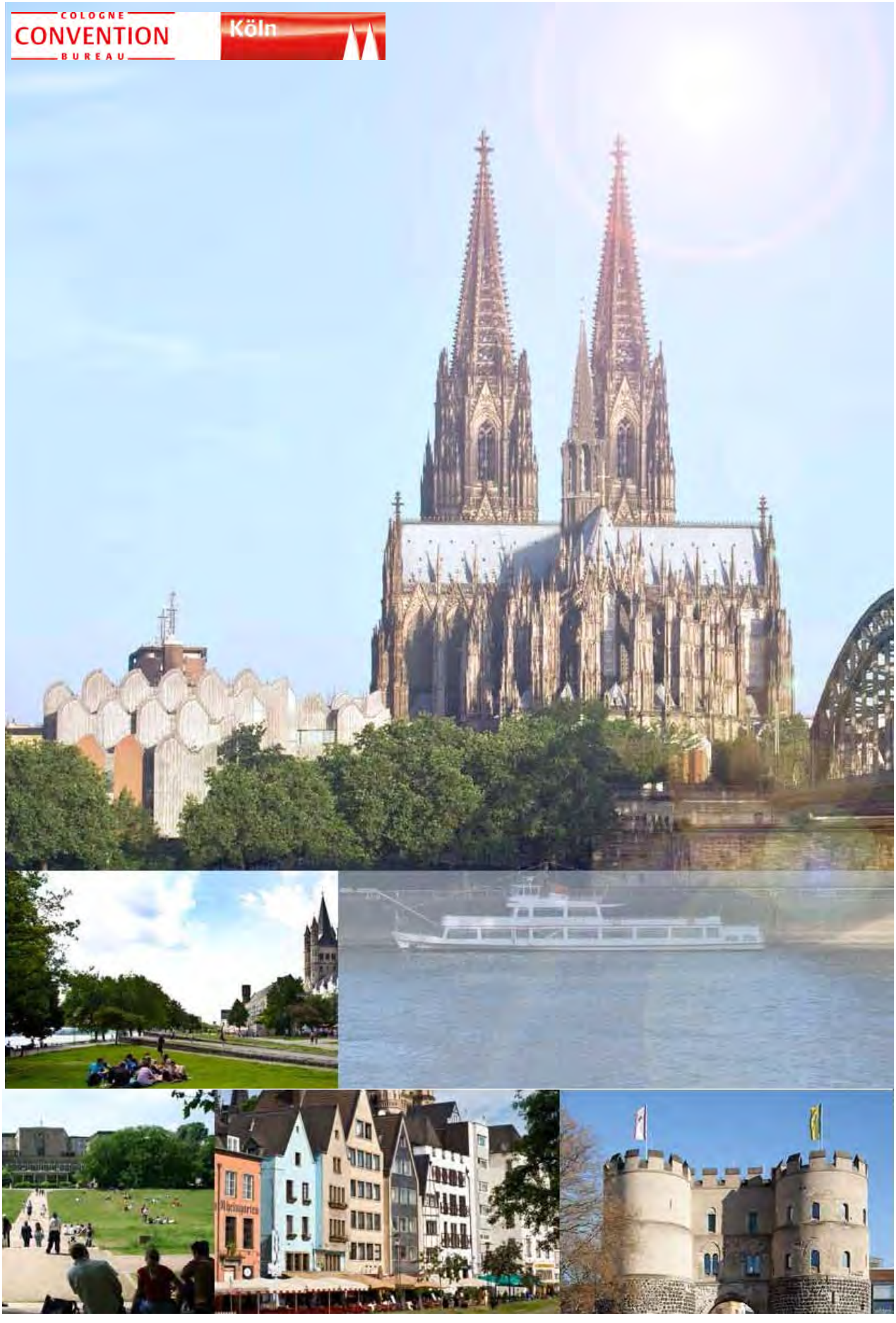
in Cologne, Germany
from October 15 - 17, 2017

Program and Poster Abstracts

University of Cologne

CMMC CENTER FOR MOLECULAR MEDICINE COLOGNE

Welcome to Cologne



Welcome to the Klenk Symposium 2017

Dear Students – Dear Colleagues – Dear Friends

We are looking forward to welcoming you to the 33rd Ernst Klenk Symposium in Molecular Medicine on "Tissue regeneration, wound healing and fibrosis: Translating basic concepts into regenerative therapy", taking place from October 15 - 17, 2017.

Tissue injury induces a complex and dynamic cellular repair program to restore normal tissue architecture and function. These repair mechanisms often require an intricate interplay between resident tissue stem cells, recruitment of inflammatory cells and the extracellular environment, and are believed to recapitulate fundamental aspects of embryonic development, organ regeneration and pathophysiology (tumorigenesis, metastasis, fibrosis) by activating similar molecular and cellular pathways. Thus, tissue repair and wound healing are crucial processes at the interface of normal tissue regeneration and development of a variety of different diseases.

Clearly, understanding the fundamental mechanisms by which these developmental programs are reactivated and maintained in adult tissues is of particular importance for the treatment of chronic injury. For example organ fibrosis - a combination of inadequate cellular function and aberrant accumulation of extracellular matrix - is a leading cause of morbidity and mortality due to diseases such as idiopathic pulmonary fibrosis, liver cirrhosis, renal fibrosis, scleroderma and myelofibrosis.

We are honored that Prof. Dr. Sabine Werner (ETH Zurich - Zurich, CH) has accepted our invitation to be the honorary guest speaker of the Ernst Klenk Lecture (October 16, 2017 at 6 p.m.) and we thank her for the support in setting-up this cutting-edge program with leading international experts.

The aim of the 33rd Klenk Symposium is to provide a platform for scientific exchange by bringing together a diverse group of scientists and physicians working at the forefront of repair and regeneration research who will share and discuss the latest advances. We would like to thank all guest speakers for their active participation and outstanding engagement.

Many thanks to all participants, who are presenting their latest research findings at the poster sessions and thereby providing an excellent opportunity for scientific exchange with over 75 posters.

The annual Klenk Symposium is a special biomedical science meeting at the University of Cologne (UoC) that attracts national and international scientists, physicians and particularly students. The meeting is named in honor of Professor Ernst Klenk, an outstanding German lipid-biochemist, who headed the Institute for Physiological Chemistry at the Medical Faculty from 1936 to 1965. Since its foundation the participation in the meeting is free of charge. Thus, we are thankful for the support provided by the German Research Foundation, our cooperating partners and industrial sponsors.

We are expecting a highly interactive meeting sparked by the panel of internationally renowned speakers, who will discuss their latest research developments with an audience of scientists and physicians.

We are looking forward to welcoming you and wish all participants an enjoyable, informative and memorable stay in Cologne.

Sincerely yours,

Prof. Dr. S. Ehrig | PD Dr. C. Niemann | Prof. Dr. Dr. T. Krieg | Prof. Dr. T. Benzing

Chairs - Cologne Klenk Symposium Board 2017

Chair - Center for Molecular Medicine Cologne

Cologne Klenk Symposium Committee 2017

Matthias Hammerschmidt - Carien Niessen - Claus Cursiefen - Bernhard Schermer - Debora Grosskopf-Kroiher - Thomas Benzing

Organization of the Ernst Klenk Symposium 2017

Center for Molecular Medicine Cologne (CMMC) - University of Cologne
CMMC-Research Building / Robert-Koch-Str. 21 /
50931 Cologne, Germany

Phone: +(49)-221-478 5552 / Fax: +(49)-221-478 4833

klenk-symposium@uni-koeln.de

www.cmmc-uni-koeln.de

The CMMC gratefully acknowledges the support by the cooperating partners

Program

Scientific Coordination

Sabine Werner ETH Zurich - Zurich, CH
and Sabine Eming • Catherin Niemann • Thomas Krieg - Cologne, DE

Cologne Ernst Klenk Symposium Committee 2017

Sabine Eming • Catherin Niemann • Thomas Krieg (Chairs)
Carien M Niessen • Matthias Hammerschmidt • Claus Cursiefen • Bernhard Schermer • Debora Grosskopf-Kroiher
Thomas Benzing

Poster Evaluation Committee 2017

Catherin Niemann (Chair)
Beate Eckes • Sabine Eming • Hamid Kashkar • Thomas Krieg • Carien M Niessen • Alvaro Rada-Iglesias
Bernhard Schermer • Gerhard Sengle • Sabine Werner

Sunday – Oct. 15, 2017

12.50 p.m. **Welcome Address**

Thomas Krieg Dean - Medical Faculty, University of Cologne

Thomas Benzing Chair - Center for Molecular Medicine Cologne, University of Cologne

Sabine Werner - Sabine Eming - Catherin Niemann - Thomas Krieg
Scientific Coordinators - Ernst Klenk Symposium 2017

Session I — Exploring molecular concepts of cell renewal and stem cell function in tissue maintenance and repair

Chairs Sabine Werner (Zurich) – Matthias Hammerschmidt (Institute for Zoology)

1.00 - 1.30 p.m. **Live imaging good and bad aspects of the wound inflammatory response**
Paul Martin - School of Medical Sciences, University of Bristol - Bristol, UK

1.30 - 2.00 p.m. **Reprogramming epigenetic memory in regeneration and cancer**
Renato Paro - ETH Zurich, Zurich and Faculty of Science, University of Basel - Basel CH

2.00 - 2.30 p.m. **From planarians to parasites: stem cells and developmental plasticity in flatworms**
Phillip A Newmark - University of Wisconsin-Madison - Madison, US

2.30 - 3.00 a.m. *Coffee Break*

Chairs Catherin Niemann (CMMC) – Alvaro Rada-Iglesias (CMMC)

3.00 - 3.30 p.m. **Stem cells and epithelial homeostasis in mammary gland development**
Ian G Macara - Vanderbilt University Sch ool of Medicine - Nashville, US

3.30 - 4.00 p.m. **Exploring stem cell fate regulation through synthetic extracellular matrices**
Matthias P Lutolf - School of Basic Science, École Polytechnique Fédérale de Lausanne - Lausanne, CH

4.00 - 4.30 p.m. **Vascular control of liver development and function**
Hellmut Augustin - German Cancer Research Center (DKFZ) - Heidelberg, DE

4.30 - 5.00 p.m. *Coffee Break*

5.00 - 8.30 p.m. **Poster session during the Welcome Get-Together (free finger food and drinks)**
Selection of three posters for the Poster Awards

Poster Presentation - Klenk Symposium 2017

We are pleased to announce that we received 77 poster abstracts related to following topics:

A 01 – A 18 Exploring molecular concepts of cell renewal & stem cell function in tissue maintenance & repair

B 01 – B 40 Mechanisms of cellular communication in tissue regeneration, repair and fibrosis

C 01 – C 09 New strategies of targeting the immune response in tissue remodeling

D 01 – D 10 Challenges and perspectives for translating scientific discoveries in regenerative medicine

The poster track is intended as a forum that provides an opportunity for the authors to present ongoing research activities/results and thereby promoting scientific interaction.

Poster Award

The Klenk Poster Award 2017 of the Center for Molecular Medicine Cologne (CMMC) will be awarded to the presenting author of the three most outstanding poster contributions. The awardees of the poster prizes are invited to give a short presentation (10 min.) during the last session on Tuesday, Oct. 17, 2017.

Monday - Oct. 16, 2017

Session II — Mechanisms of cellular communication in tissue regeneration, repair and fibrosis

Chairs	Beate Eckes (Dept. of Dermatology) – Gerhard Sengle (Institute for Biochemistry II)
9.00 - 9.30 a.m.	Scar Wars: fibroblast lineages and fibrosis Michael T Longaker - Stanford University School of Medicine - Stanford, US
9.30 - 10.00 a.m.	Mechanotransduction and cell-matrix interaction regulates collective cell migration Joachim P Spatz - Max-Planck-Institute for Medical Research - Heidelberg, DE
10.00 - 10.30 a.m.	Muscle stem cell regenerative decline with aging Pura Munoz-Canoves - ICREA and Pompeu Fabra University - Barcelona, ES
10.30 - 11.00 a.m.	*Coffee Break*
Chairs	Cornelia Mauch (Dept. of Dermatology) – Reinhard Büttner (Institute for Pathology)
11.00 - 11.30 a.m.	Do fibroblast growth factors regulate fibrosis? David M Ornitz - Washington University School of Medicine - St. Louis, US
11.30 - 12.00 a.m.	p53 at the junction of tissue regeneration and cancer Raffaella Sordella - Cold Spring Harbor Laboratory - Cold Spring Harbor, US
12.00 - 12.30 p.m.	Multistep cancer stromal cell activation and evolution Gian-Paolo Dotto - University of Lausanne - Lausanne, CH
12.30 - 2.00 p.m.	*Lunch Break with Poster Presentation (free soup and sandwiches for all participants)*

Session III — New strategies of targeting the immune response in tissue remodeling

Chairs	Martin Krönke (Vice Chair of the CMMC) – Sabine Eming (Dept. of Dermatology)
2.00 - 2.30 p.m.	Type 2 immunity in tissue regeneration and fibrosis Thomas A Wynn - National Institute of Allergy and Infectious Diseases, NIH - Bethesda, US
2.30 - 3.00 p.m.	Immune cell-adipose tissue interactions during infection Edward J Pearce - Max-Planck-Institut für Immunbiologie und Epigenetik - Freiburg, DE
3.00 - 3.30 p.m.	Imaging the role of innate immunity in tissue repair Paul Kubes - University of Calgary - Calgary, CA
3.30 - 3.50 p.m.	*Coffee Break*
Chairs	Manolis Pasparakis (CECAD Cologne) – Hamid Kashkar (IMMHI)
3.50 - 4.00 p.m.	Announcement of the Poster Awards - Sabine Eming and Catherin Niemann
4.00 - 4.30 p.m.	Development of hepatoprotective and pro-regenerative strategies: learning from nature Matias A Avila - Center for Applied Medical Research, University of Navarra - Pamplona, ES
4.30 - 5.00 p.m.	Immune Strategies for accelerating regeneration Nadia A Rosenthal - The Jackson Laboratory - Bar Harbor, US
5.00 - 5.30 p.m.	On the role of the immune system in supporting and fighting liver damage and liver cancer Mathias Heikenwälder - German Cancer Research Center (DKFZ) - Heidelberg, DE
5.30 - 6.00 p.m.	*Coffee Break*

Ernst Klenk Lecture: 6.00 - 7.00 p.m.

How to control your neighbor – stromal epithelial cross-talk in tissue repair and inflammatory disease

Sabine Werner - Institute for Molecular Health Sciences, ETH Zurich - Zurich, CH

Laudation: Thomas Krieg

Tuesday - Oct. 17, 2017

Session IV	Challenges and perspectives for translating scientific discoveries in regenerative medicine
Chairs	Carien Niessen (CECAD Cologne) – Claus Cursiefen (Dept. of Ophthalmology)
9.00 - 9.30 a.m.	Cell and tissue therapy. Advances in science and practice Robert S Kirsner - University of Miami Miller School of Medicine - Miami, US
9.30 - 10.00 a.m.	Developing induced pluripotent stem (iPS) cell-based therapy for inherited skin diseases Dennis R Roop - School of Medicine, University of Colorado Anschutz Medical Campus - Aurora, US
10.00 - 10.30 a.m.	GPS: Navigating the pathway for regenerative therapy Robert Sackstein - Brigham and Women's Hospital and Harvard Medical School - Boston, US
10.30 - 11.00 a.m.	*Coffee Break*
Chairs	Thomas Benzing (Chair of the CMMC) – Björn Schumacher (CECAD Cologne)
11.00 - 11.30 a.m.	ES Tumor associated macrophages: from mechanism to therapy Jeffrey W Pollard - University of Edinburgh - Edinburgh, UK
11.30 - 12.00 p.m.	Regenerating the damaged heart by stimulating cardiomyocyte proliferation Jeroen Bakkers - Hubrecht Institute, University Medical Centre Utrecht - Utrecht, NL
12.00 - 12.30 p.m.	CRISPR-Cas9 genome editing, from mechanism to therapy Jacob E Corn - Dept. of Molecular and Cell Biology and Innovative Genomics Institute, University of California - Berkeley, US
12.30 - 1.00 p.m.	Short presentation given by the three poster awardees (3 x 10 min. short talk)
1.00 - 1.15 p.m.	Summary, open questions, concluding remarks Sabine Werner - Sabine Eming - Catherin Niemann - Thomas Krieg

General Information

The meeting is open and no registration or attendance fee is required.
Participation is free of charge - free registration is required.

Childcare is provided by Spielland, University Hospital Cologne.

If you require an official invitation letter for travel refunding or visa issues, please contact
Debora Grosskopf-Kroiher - debora.grosskopf-kroiher@uni-koeln.de

Center for Molecular Medicine Cologne (CMMC), University of Cologne
CMMC Research Building, Robert-Koch-Str. 21, 50931 Cologne, Germany

Venue

Main Lecture Hall, Faculty of Medicine, University of Cologne
Access only possible from Joseph-Stelzmann-Str. 26 - please follow the yellow Klenk signs.
http://www.cmmc-uni-koeln.de/events/ernst-klenk-symposium/ernst-klenk-symposium-2017/venue/

We gratefully acknowledge support by the

DFG Deutsche Forschungsgemeinschaft

We gratefully acknowledge support by following industrial sponsors

Notes

Poster Abstracts

Overview of the poster abstracts

	First Author	E-Mail	Affiliation (first affiliation listed)	Poster Title	PP
A-01	Bertozzi, A	alberto.bertozzi@uni-ulm.de	Institute for Biochemistry and Molecular Biology, Ulm University, Ulm, DE	Zebrafish can fully regenerate the heart by quantitative restoration of cardiomyocyte number	13
A-02	Chacón-Martínez, C A	carlos.chacon@age.mpg.de	Max Planck Institute for Biology of Ageing, Cologne, Cologne, DE	An *in vitro* reconstituted stem cell niche reveals dynamic cell fate transitions between stem cells and their progeny	14
A-03	Dalvoy, M	mohan.dalvoy@uni-ulm.de	Institute of Biochemistry and Molecular Biology, Ulm University, Ulm, DE	Cardiomyocyte proliferation requires resolution of genomic stress by BMP signaling during zebrafish heart regeneration	15
A-04	Ding, X	xiaolei.ding@uk-koeln.de	Dept. of Dermatology, University of Cologne, Cologne, DE	Identification of essential and unique functions of mTOR signaling in skin development and homeostasis	16
A-05	Geueke, A	ageueke@uni-koeln.de	Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, DE	Mechanisms of stem cell apoptosis in epidermal regeneration	17
A-06	Giriyapura, B	bharath.giriyapura@uk-koeln.de	Molecular Cell Biology, Institute for Anatomy, Univ. of Cologne, Cologne, DE	Functional analysis of *mthl7* and *mthl8* in context of aging and stemness in the female germline stem cells of *Drosophila melanogaster*	18
A-07	Göbel, K	klaus.goebel@uni-koeln.de	Cologne Excellence CECAD), Univ. of Cologne, Cologne, DE	Cellular polarity and metabolic signalling in sebaceous gland homeostasis	19

	First Author	E-Mail	Affiliation (first affiliation listed)	Poster Title	PP
A-08	Joost, S	tina.jacob@ki.se	Center for Innovative Medicine and Dept. of Biosciences and Nutrition, Karolinska Inst., Stockholm, SE	Plasticity, convergence and legacy of Lgr5 and Lgr6 stem cells during cutaneous wound repair	20
A-09	Kimoloi, S	obaris@uni-koeln.de	Institute of Vegetative Physiology, Univ. of Cologne, Cologne, DE	Mitochondrial dysfunction in muscle stem cells impairs regeneration	21
A-10	McGinn, J	mpa28@cam.ac.uk	Wellcome Trust/ Med. Research Council Cambridge Stem Cell Inst., Univ. of Cambridge, Cambridge, UK	Epithelial stem cell fate plasticity: a mouse oesophageal model	22
A-11	Nafisi, J	jnafisi@smail.uni-koeln.de	Dept. of Derma-tology, Univ. of Cologne, Cologne, DE	Polarity signaling in the regulation of spindle orientation	23
A-12	Philipp, D	denise.philipp@uk-koeln.de	Dept. of Cardio-thoracic Surgery, Heart Center, Univ. of Cologne, Cologne, DE	Pre-activated murine bone marrow-derived mesenchymal stem cells drive M2b polarization	24
A-13	Rübsam, M	ruebsam0@uni-koeln.de	Dept. of Derma-tology, Univ. of Cologne, Cologne, DE	E-cadherin controls EGFR signaling and tissue tension for polarized epidermal junction and barrier formation	25
A-14	Rust, K	krust@uni-koeln.de	Inst. I for Anatomy, Univ. of Cologne, Cologne, DE	A Myc-Tip60 transcriptional network maintains *Drosophila* neural stem cells by regulating the polarity determinant aPKC	26
A-15	Schlusche, A K	igor.jakovcevski@uk-koeln.de	Inst. for Molecular and Behavioral Neuroscience, Univ. of Cologne, Cologne, DE	Embryonic loss of HCN/h current impedes development of the cerebral cortex by reducing neural stem cell proliferation	27

	First Author	E-Mail	Affiliation (first affiliation listed)	Poster Title	PP
A-16	Steens, J	jennifer.steens@uk-essen.de	Inst. of Cell Biology, Univ. Hospital Essen, Medical Faculty, Univ. of Duisburg-Essen, Essen, DE	From murine induced pluripotent stem cells (iPSCs) cells to vascular wall-typical mesenchymal stem cells (VW-MSCs) – using a VW-MSC specific Gene-code	28
A-17	Széky, B	szekyb@gmail.com	Dept. of Dermatology, Venereology & Dermatooncology, Semmelweis Univ., Budapest, HR	Analysis of Dermal Stem Cells in Healthy and Diseased Skin	29
A-18	Wachsmuth, E	ewachsm1@uni-koeln.de	Dept. of Dermatology, Univ. of Cologne, Cologne, DE	Disturbed metabolic regulation of skin barrier function in hyperglycemic and type 2 diabetes	30
B-01	Bhavsar, M	mbhavsa@gwdg.de	Exp. Orthopedics and Trauma Surgery, J.W. Goethe-Univ. Frankfurt, Frankfurt am Main, DE	Role of endogenous bioelectric currents in controlling cell differentiation	33
B-02	Delii, V	oksana.sulaieva@gmail.com	Dept. of Pathology, Ukrainian Research and Practical Center of Endocrine Surgery, Kiev, UA	Possible mechanisms of peptic ulcer non-healing after bleeding	34
B-03	Fearon, A	abbie.fearon@biol.ethz.ch	Inst. of Molecular Health Sciences, ETH Zürich, Zurich, CH	The RNA methyltransferase Nop2 is a novel regulator of liver regeneration	35
B-04	Fischer, B	bfischer@hdz-nrw.de	Heart and Diabetes Center NRW, Ruhr-Univ. Bochum, Bad Oeynhausen, DE	Treatment with 4-Methyl-umbelliferyl-β-D-xyloside and uridine diphosphate leads to an increased intracellular XT-activity in normal human dermal fibroblasts	36
B-05	Frech, S	beate.lichtenberger@meduniwien.ac.at	Dept. of Dermatology, Medical University of Vienna, Vienna, AU	Fibroblast heterogeneity in wound healing and fibrosis	37

	First Author	E-Mail	Affiliation (first affiliation listed)	Poster Title	PP
B-06	Géraud, C	johanna.zierow@medma.uni-heidelberg.de	Dept. of Dermatology, Venereology and Allergology, Univ. Med. Center & Med. Faculty Mannheim, Heidelberg Univ., Mannheim, DE	GATA4-dependent organ-specific endothelial differentiation controls liver development and embryonic hematopoiesis	38
B-07	Godoy, P	godoy@ifado.de	IfADo-Leibniz Research Centre for Working Environment and Human Factors, TU Dortmund, Dortmund, DE	Transcriptomic analysis of acute and chronic liver injury reveals an inflammation / ER stress dependent suppression of metabolic gene networks	39
B-08	González, D	gonzalez@ifado.de	Leibniz Research Centre for Working Environment and Human Factors, TU Dortmund, Dortmund, DE	WISP1: A potential modulator of inflammatory responses in acute liver injury	40
B-09	Goren, I	goren@chemie.uni-frankfurt.de	*pharmazentrum frankfurt*/ZAFES, Klinikum der Johann Wolfgang Goethe - Universität, Frankfurt, DE	Anti-inflammatory effects of rosiglitazone in obesity-impaired wound healing depend on adipocyte differentiation	41
B-10	Harmanci, D	duyguharmanci@gmail.com	Dept. of Molecular Medicine, Graduate School of Health Sciences, Dokuz Eylul Univ., Izmir, TR	MiR-29b-2 reduces Lysyl oxidase activity in primary hypertrophic scar cells	42
B-11	Hesse, C	christina.hesse@item.fraunhofer.de	Fraunhofer Inst. for Toxicology and Experimental Medicine, Hanover, DE	Intact cell-cell interactions in fresh *ex vivo* lung tissue enables the investigation of pro-fibrotic mediators	43
B-12	Hiebert, P	paul.hiebert@biol.ethz.ch	Institute of Molecular Health Sciences, ETH Zürich, Zurich, CH	Nrf2-mediated fibroblast reprogramming drives cellular senescence by targeting the matrisome	44
B-13	Hörst, K	katharina.hoerst@fu-berlin.de	Institute of Pharmacy, Department of Pharmacology and Toxicology, Freie Univ. Berlin, Berlin, DE	Regenerative potential of adipocytes – Potential treatment for hypertrophic scars?	45

	First Author	E-Mail	Affiliation (first affiliation listed)	Poster Title	PP
B-14	Jahn, C	christopher.jahn@uni-ulm.de	Institute of Biochemistry and Molecular Biology, Ulm University, Ulm, DE	Tissue-specific roles of FGF signaling during zebrafish caudal fin regeneration	46
B-15	Jain, M	manaswita.jain@uk-koeln.de	Dept. II of Internal Medicine, Univ. of Cologne, Cologne, DE	Loss of AATF in renal epithelial cells results in accumulation of DNA damage, defective primary cilia and a degenerative kidney disease resembling human Nephronophthisis	47
B-16	Jordan, N	nina.jordan@newcastle.ac.uk	Institute of Cellular Medicine, Newcastle University, Newcastle, UK	Preventing endothelial-to-mesenchymal transition by enhancing miR126-3p expression	48
B-17	Ketteler, J	julia.ketteler@uk-essen.de	Institute of Cell Biology, Univ. of Duisburg-Essen, Essen, DE	How stromal fibroblasts foster radiation resistance in human prostate cancer xenografts	49
B-18	Klenner, F	felix.klenner@uni-ulm.de	Inst. of Biochemistry and Molecular Biology, Ulm University, Ulm, DE	Generic wound signals initiate regeneration in a missing-tissue context	50
B-19	Kocak, A	kocak.ayse@gmail.com	Dept.of Molecular Medicine, Graduate School of Health Sciences, Dokuz Eylul University, Izmir, TR	Epigallocatechin-3-gallate protects against oxidative stress in scleroderma animal model	51
B-20	Köhler, A	a.koehler@uni-koeln.de	Center for Biochemistry, Univ. of Cologne, Cologne, DE	Ablation of epidermal collagen chaperoning results in dermal fibrosis	52
B-21	Marcher, A	ravnskjaer@bmb.sdu.dk	Dept. of Biochemistry and Molecular Biology, Univ. of Southern Denmark, DK	Identification of NASH-associated signaling networks in murine hepatic stellate cells	53

	First Author	E-Mail	Affiliation (first affiliation listed)	Poster Title	PP
B-22	Moeller, K	katharina.moeller@uk-koeln.de	Department of Anaesthesiology and Intensive Care, Univ. Hospital of Cologne, Cologne, DE	Collagens modulate sensitization signaling and CGRP expression of nociceptive neurons	54
B-23	Mohamed, F	fatma.mohamed@medma.uni-heidelberg.de	Dept. of Medicine II, Medical Faculty Mannheim, Heidelberg Univ., Mannheim, DE	Cellular expression of TLR2 is positively associated with cellular proliferation and apoptosis as well as VEGF expression in HCC	55
B-24	Neumann, S	maike.kuemper@uk-koeln.de	Department of Dermatology, Univ. of Cologne, Cologne, DE	Endothelial cell-derived MMP-14 is dispensable for skin formation and repair	56
B-25	Nüchel, J	nuechel.julian@uni-koeln.de	Institute for Biochemistry II, Univ. of Cologne, Cologne, DE	Fibroblast secretion of TGFβ1 is executed by secretory autophagy	57
B-26	Owlarn, S	suthira.owlarn@mpi-muenster.mpg.de	Max Planck Res. Group Stem Cells and Regeneration, Inst. for Molecular Biomedicine, Univ. of Münster, Munster, DE	Generic wound signals initiate regeneration in missing-tissue contexts	58
B-27	Pach, E	elke.pach@uk-koeln.de	Dept. of Dermatology, Univ. of Cologne, Cologne, DE	Role of fibroblast MMP-14 in skin homeostasis and melanoma growth	59
B-28	Pantasis, S	sophia.pantasis@biol.ethz.ch	Institute of Molecular Health Science, ETH Zürich, Zurich, CH	Function of the secreted tyrosine kinase VLK in liver injury and regeneration – Extracellular phosphorylation as a potential regulatory mechanism of matrix remodeling	60
B-29	Peters, F	fpeters@biochem.uni-kiel.de	Biochemical Institute, Christian-Albrechts-Univ. of Kiel, Kiel, DE	Role of astacin-like proteinases in wound healing, scarring and fibrosis	61

	First Author	E-Mail	Affiliation (first affiliation listed)	Poster Title	PP
B-30	Pincha, N	npincha@gmail.com	IFOM, Centre for Inflammation and Tissue Homeostasis, Bangalore,IN	PAI-1 mediates mast cell-fibroblast interactions in a mouse model of fibrosis	62
B-31	Plotczyk, M	magdalena.plotczyk16@imperial.ac.uk	Department of Bioengineering, Imperial College London, London, UK	The role of hair follicles in dermal remodelling and scar reduction	63
B-32	Saupe, S	susann.saupe@medizin.uni-leipzig.de	Department of Dermatology, Allergology and Venerology, Leipzig Univ. Medical Center, Leipzig, DE	Regulation of macrophage polarization by fibroblasts	64
B-33	Schmidt, K G	k.schmidt@med.uni-frankfurt.de	*pharmazentrum frankfurt*/ZAFES, Inst. of Pharmacology & Toxicology, Hospital - Goethe-Univ., Frankfurt, Frankfurt, DE	Sphingosine -1- phosphate receptor 5 modulates early stage processes of fibrogenesis in a mouse model of scleroderma: a pilot study	65
B-34	Slaats, G	gisela.slaats@uk-koeln.de	Dept. II of Internal Medicine, Univ. of Cologne, Cologne, DE	Loss of *Ercc1* in renal tubular epithelial cells results in degenerative kidney disease and renal fibrosis	66
B-35	Verboven, E	elisabeth.verboven@kuleuven.vib.be	Centre for Cancer Biology, VIB-KU Leuven, Leuven, BE	The role of *Hippo* signalling in liver regeneration	67
B-36	Wang, L	lwang7@smail.uni-koeln.de	Inst. for Pathology, Univ. Hospital of Cologne, Cologne, DE	Epigenetic function of the lysine specific demethylase LSD1 in hepatic stellate cell activation during liver fibrosis	68
B-37	Wietecha, M S	mateusz.wietecha@biol.ethz.ch	Institute of Molecular Health Sciences, Department of Biology, ETH Zürich, Zurich, CH	Transcriptomic meta-analysis of healing wounds, tumor stroma, wound-derived fibroblasts and cancer-associated fibroblasts identifies phase-specific gene signatures, which categorize tumor fibroblasts into wound-associated phenotypes	69

	First Author	E-Mail	Affiliation (first affiliation listed)	Poster Title	PP
B-38	Wirtz, L	lisa.wirtz@uni-koeln.de	Center for Biochemistry, Univ. of Cologne, Cologne, DE	Does the collagen-matrix provide a mechanical barrier for efficient entry of herpes simplex virus 1 into the dermis?	70
B-39	Yao, W	yao.wenjie@uk-koeln.de	Center for Molecular Medicine Cologne, Univ. of Cologne, Cologne, DE	Molecular basis of controlled *in vivo* cellular reprogramming that enables cardiac regeneration	71
B-40	Zuk, A	cadamo1@uni-koeln.de	Center for Biochemistry, Univ. of Cologne, Cologne, DE	Fibrillin-1 controls dermal fibrosis	72
C-01	Belemets, N	oksana.sulaieva@gmail.com	Department of Fundamental Medicine, Taras Shevchenko National Univ. of Kiev, Kiev, UA	Heterogeneity of adipose tissue remodeling in rats with obesity induced by monosodium glutamate	75
C-02	Garcia, J	john.garcia@rjah.nhs.uk	Keele Univ., Robert Jones & Agnes Hunt Orthopaedic Hospital, Oswestry, UK	Characterization of joint macrophages in early and end stage osteoarthritis	76
C-03	Hou, Y	yhou@smail.uni-koeln.de	Dept. of Ophthalmology, University of Cologne, Cologne, DE	Photodynamic therapy leads to time dependent regression of pathological corneal (lymph)angio-genesis and promotion of high-risk corneal graft survival	77
C-04	Kühn, S	kuehn-sebastian@ipfdd.de	Leibniz Inst. of Polymer Research Dresden, Dresden, DE	StarPEG-heparin microgels – customised materials for promotion of wound healing	78
C-05	Mori, R	ryoichi@nagasaki-u.ac.jp	Dept. of Pathology, Nagasaki University School of Medicine, Nagasaki, JP	Comprehensive identi-fication of wound healing and inflammation miRNAs reveals a key role for miR-223 in neutrophilic clearance of *Staphylo-coccus aureus* at wound sites	79

	First Author	E-Mail	Affiliation (first affiliation listed)	Poster Title	PP
C-06	Schuster, R	ronensc@post.bgu.ac.il	Dept. of Clinical Biochemistry and Pharmacology, Ben Gurion Univ. of the Negev, Negev, IL	Modulating NF-kB signaling in the early wound as a means to accelerate tissue repair	80
C-07	Umehara, T	umehara@nagasaki-u.ac.jp	Division of Forensic Pathology and Science, Unit of Social Medicine, Course of Medical and Dental Sciences, Nagasaki Univ. School of Med., Nagasaki, JP	Identification of specific microRNAs in diabetic-derived neutrophils: functional analysis of miR-129-2-3p and inflammation-related genes	81
C-08	Vulovic, M	igor.jakovcevski@uk-koeln.de	Dept. of Anatomy and Forensic Medicine, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, RS	Impact of depletion of microglia/macrophages on regeneration after spinal cord injury	82
C-09	Willenborg, S	sebastian.willenborg@uk-koeln.de	Department of Dermatology, Univ. of Cologne, Cologne, DE	Novel insights in the link between type 2 innate signals and initiation of pro-fibrotic pathways	83
D-01	Akpulat, U	sebahattin.cirak@uk-koeln.de	Center for Molecular Medicine Cologne (CMMC), Univ. of Cologne, Cologne, DE	Antisense morpholino oligonucleotides enter dystrophic muscle fibres via myoblast cell fusion *in-vivo* and provide a rational approach for efficient dystrophin restoration in Duchene Muscular Dystrophy	85
D-02	Atallah, P	atallah@ipfdd.de	Max Bergmann Center of Bio-materials, Leibniz Institut für Polymer-forschung, Dresden, Dresden, DE	Tailored sulfation patterns of heparin in biohybrid hydrogels for modulating cytokine signals by wound dressings	86
D-03	Cherepkova, E V	india06@mail.ru	Budgetary State-Research Institute of Physiology and Basic Medicine, Novosibirsk, RU	Vascular endothelium dysfunction in the patients with solid malignancies and their regression following the radical surgery	87

	First Author	E-Mail	Affiliation (first affiliation listed)	Poster Title	PP
D-04	Gabriel, E	egabriel@uni-koeln.de	Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, DE	Retinal tissue engineering in the dish: from disease mechanisms to personalized regenerative medicine	88
D-05	Lenger, K	dr.karin.lenger@t-online.de	Institute for Scientific Homeopathy, Offenbach, DE	Wound healing is cured by magnetic photons detected in homeopathic remedies	89
D-06	Li, X	ning.xu@ki.se	Department of Medicine Solna, Karolinska Institutet, Stockholm, SE	MicroRNA-132, a promising target for wound therapy	90
D-07	Oliveira, K M C	karlamychellyne@yahoo.com.br	Frankfurt Initiative for Regenerative Medicine (FIRM), J. W. Goethe-Univ. of Frankfurt, Frankfurt, DE	Histological characterization of traumatic neuroma development	91
D-08	Poosti, F	fariba.poosti@kuleuven.be	Dept. Pathology and Medical Biology, Univ. Medical Center Groningen, Univ. of Groningen, Groningen, NL	Selective targeting of Fibroferon to interstitial myofibroblasts attenuates renal fibrosis	92
D-09	Rusek, M	marta.rusek@yahoo.com	Dept. of Dermatology, Venereology and Pediatric Dermatology, Medical Univ. of Lublin, Lublin, PL	MiRNAs regulation and its role as biomarkers in scleroderma	93
D-10	Yuan, X	xiaodong.yuan@medma.uni-heidelberg.de	Dept. of Medicine II, Medical Faculty Mannheim, Heidelberg Univ., Mannheim, DE	Transcription factor complex TRIM33-Smad2/3 is crucial for progenitor cell-mediated liver regeneration in liver cirrhosis	94

Information “Poster Presentation”

Poster size
DIN A0 format (84 cm wide and 118 cm high).

Poster board / mounting and poster display
Please refer to your abstract in the poster abstract book for the poster board number (PBN) assigned to you. Please use the board with the same number displayed in the upper right corner of the poster board. At the registration desk you will receive the location of the poster sessions as well as push pins and tape upon arrival at the symposium.

Poster Sessions
The poster presentation will take place:

Sunday, Oct. 15, 2017, from 5.00 - 8.30 p.m.
during the Welcome Get-Together as follows:

- 5.30 - 6.30 p.m.: poster presentation A, C and D
- 6.30 - 7.30 p.m.: poster presentation B
- 7.30 - 8.30 p.m.: ongoing discussions

Monday, Oct. 16, 2017 from 12.30 - 2.00 p.m.
during the lunch break at the venue

In order to share the novel and exciting ongoing state-of-the-art research with all participants and thereby promoting scientific interaction, the poster should be displayed during the entire duration of the symposium. The Symposium ends on Tuesday, Oct. 17, 2017 at about 1.15 p.m.

Poster Awards
The Poster Evaluation Committee (PEC) will judge posters on the scientific content with a focus on originality, potential impact/importance of the topic, novelty and relevance. The PEC decided to award 250,- Euro to the first author of the three most outstanding poster contributions. The *CMMC Klenk Symposium Poster Awards* will be presented by Catherin Niemann (Chair of PEC) on Monday, Oct. 16, 2017 at 3.50 p.m.

Tuesday, Oct. 17, 2017 during session IV of the Klenk Symposium 2017
Awardees of the poster prizes are invited to give a short presentation (10 min.)

Further Information
If you have further questions, please contact Debora Grosskopf-Kroiher (debora.grosskopf-kroiher@uni-koeln.de).

Zebrafish can fully regenerate the heart by quantitative restoration of cardiomyocyte number

A. Bertozzi[1] / M. D. Vasudevarao[1] / G. Weidinger[1]

Institute for Biochemistry and Molecular Biology, Ulm University, Ulm, Germany

alberto.bertozzi@uni-ulm.de

Introduction

In adult mammals, cardiac infarction results in permanent functional impairments and thus can lead to heart failure. Injured myocardial tissue forms a permanent, collagen-rich scar and damaged cardiomyocytes can largely not be replaced. In contrast, zebrafish appear to be able to repair heart injuries in response to apical resection, tissue necrosis caused by cryoinjury and cardiomyocyte death caused by genetic ablation. In all cases, the injured area matures within several days into a complex, collagen- and fibrin-rich tissue. However, in contrast to the situation in mammals, this scar is transient, since it is typically reabsorbed during cardiac repair in zebrafish; in fact, little or no collagen is retained by 1–2 months after injury and permanent scars do not form. In response to injury, cardiomyocytes re-enter the cell cycle and it is generally assumed that lost cardiomyocytes (CMs) are replaced by proliferation of spared CMs. However, it has actually remained unclear whether the myocardium fully regenerates, that is whether the pre-injury number of CMs is restored.

First, currently available lineage-tracing techniques cannot distinguish between pre-existing and regenerated myocardium, thus the amount of regenerated tissue cannot easily be determined. Secondly, some studies using measurements of heart function in regenerated hearts have hinted at incomplete regeneration. Thirdly, while morphological regeneration defined by the complete resorption of wound tissue takes 30 to 90 days depending on the type of injury, significant CM cell cycle activity only occurs during the first two weeks after injury. Taken together, these evidences raise the possibility that only a fraction of the pre-injury CMs is restored and thus myocardial regeneration is not complete in zebrafish.

Results

To address this issue, we developed a method for counting the number of CMs on cryosections of myl7:nDsRed transgenic hearts, which is based on stereological principles and automated image analysis. We counted the number of CMs in a large pool of animals at 3 and 90 days post sham-surgery or injury (dpi). At 3 dpi, on average 30 % of CMs had been lost to cryoinjury.

Intriguingly, at 90 dpi the CM number did not differ between the sham-operated and injured experimental groups, indicating that CMs are indeed fully restored upon regeneration. At 90 dpi, 33 % of hearts still contained wound tissue, but the CM number in wound-containing and morphologically normal hearts was not different. Intriguingly, in the former group, CMs that constitute the compact layer around the wound appear to have a higher density compared to CMs far from the injured area.

Perspectives

These data suggest that CMs can regenerate their original number independently from the presence of any remaining scar, forming a thick and dense compact layer that encloses the remaining wound. Thus, wound closure and scarring absorption seem to be processes uncoupled from CM proliferation. Our results establish that zebrafish hearts fully regenerate the myocardium and show that wound size measurements are of limited use to assess the extent of CM regeneration.

An ***in vitro*** reconstituted stem cell niche reveals dynamic cell fate transitions between stem cells and their progeny

C. A. Chacón-Martínez[1] / M. Klose[2] / C. Niemann[3,4] / I. Glauche[2] / S. A. Wickström[1,5]

[1]Paul Gerson Unna Group 'Skin Homeostasis and Ageing', Max Planck Institute for Biology of Ageing, Cologne, Germany
[2]Institute for Medical Informatics and Biometry, Carl Gustav Carus Faculty of Medicine, Technische Universität Dresden, Germany
[3]Institute for Biochemistry II, Medical Faculty, University of Cologne, Germany
[4]Center for Molecular Medicine Cologne, University of Cologne, Germany
[5]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Germany

carlos.chacon@age.mpg.de

The epidermis is a self-renewing organ maintained by stem cells (SCs). SC fate must be tightly regulated to ensure maintenance of a stable pool of SCs, while generating differentiated progeny. Failure to maintain this balance leads to pathologies such as premature ageing and cancer.

We have, for the first time, established an *ex vivo* culture system that allows expansion and long-term maintenance of hair follicle SCs (HFSCs) without loss of their transcriptional identity and multipotency[1].

Our system recapitulates the recently discovered *in vivo* reprograming of non-SCs to SCs, and enables manipulation and interrogation of dynamic fate decisions of SCs and their progeny. This system represents an unprecedented tool to understand how SC fate is regulated and it will benefit basic as well as translational skin research.

References

1. Chacón-Martínez, C.A., Klose, M., Niemann, C., Glauche, I., Wickström, S.A. 2017. Hair follicle stem cell cultures reveal self-organizing plasticity of stem cells and progeny. ***EMBO J***. Jan 17; 36(2):151-164. e201694902.

Cardiomyocyte proliferation requires resolution of genomic stress by BMP signaling during zebrafish heart regeneration

M. Dalvoy / C. C. Wu / K. Schnabel / G . Weidinger

Institute of Biochemistry and Molecular Biology, Ulm University, Germany

mohan.dalvoy@uni-ulm.de

Introduction

In contrast to mammals zebrafish can efficiently regenerate their hearts. Cardiomyocyte regeneration occurs via proliferation of differentiated cardiomyocytes. However, cell cycle re-entry of non-cycling cells could result in replication stress and DNA damage, a phenomena known to be a hindrance to homeostasis and regeneration in mammals.

Results

Surprisingly, we found that a large fraction of proliferating cardiomyocytes in regenerating zebrafish hearts accumulate the phosphorylated histone variant H2a.x (gammaH2a.x), a marker of DNA damage. The temporal profile of DNA damage in cardiomyocytes closely followed that of cell cycle re-entry, while gammaH2a.x accumulation was not observed during physiological heart growth. EdU incorporation experiments showed that majority of all cardiomyocytes that become gammaH2a.x positive had entered the cell cycle. Thus, we hypothesize that cardiomyocytes experience replication stress during heart regeneration. Nevertheless, regeneration proceeds and cardiomyocytes can proliferate, implying that zebrafish hearts possess efficient mechanisms of alleviating genomic stress. Indeed we found that inhibition of DNA damage response pathways resulted in reduction of regenerative cardiomyocyte proliferation.

We have recently shown that BMP signaling is activated in cardiomyocytes at the wound border and that BMP signaling is required for heart regeneration by promoting cardiomyocyte proliferation (Wu et al. Dev Cell 2016). Since BMP signaling is not required for cardiomyocyte proliferation during physiological heart growth, it might regulate regeneration-specific cellular processes in cardiomyocytes, which are prerequisites for proliferation. Intriguingly, we found that inhibition of BMP signaling increased the number of gammaH2a.x positive cardiomyocytes, while pathway overactivation alleviated DNA damage, suggesting that BMP signaling is required for protection from replication stress or repair of DNA damage. Moreover, activation of BMP signaling could also resolve hydroxyurea induced exogenous replicative stress and rescued cells from cell cycle arrest.

Conclusion

Our results indicate that young zebrafish experience genomic stress during regeneration and that their ability to completely regenerate the heart could be due to an enhanced ability to resolve genomic stress. DNA damage is thought to be one of the mechanisms limiting regeneration in mammalian tissues. We propose that BMP signaling promotes regeneration either by protecting cardiomyocytes from replicative stress or by enhancing the resolution of DNA damage, a novel role for this pathway which could be conserved in other species.

Identification of essential and unique functions of mTOR signaling in skin development and homeostasis

X. Ding[1] / W. Bloch[2] / S. Iden[3,4] / M. Rüegg[5] / M. Hall[5] / M. Leptin[3,6] / L. Partridge[4,7] / S. Eming[1,3,4]

[1]Department of Dermatology, University of Cologne, Germany
[2]Department of Molecular and Cellular Sport Medicine, German Sport University Cologne, Germany
[3]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Germany
[4]CECAD, University of Cologne, Germany
[5]Biozentrum, University of Basel, Switzerland
[6]Institute for Genetics, University of Cologne, Germany
[7]Max Planck Institute for Biology of Ageing, Cologne, Germany

xiaolei.ding@uk-koeln.de

Introduction

The epidermis serves as primary interface between the body and its environment and protects the organism from dehydration and external insult. As a stratified squamous epithelium the epidermis fulfils its function through a lifelong self-renewal process that is precisely coordinated by regenerative pathways, which in part recapitulate those that are activated in epidermal morphogenesis. The exact mechanisms that orchestrate the fine-tuned balance between progenitor cell division in the epidermal basal layer and terminal differentiation of daughter cells within suprabasal layers remain to be determined. Mammalian target of rapamycin (mTOR) kinase mediates its function through two distinct multiprotein complexes, mTOR complex 1 (mTORC1) and mTORC2. mTOR signaling senses and integrates environmental cues from nutrients and growth factors, acting as important nexus in controlling cell growth and metabolism. In this study we hypothesized mTOR a critical role of the in epidermal barrier formation and homeostasis.

Results

To determine the role of mTOR signaling in epidermal development and homeostasis we specifically disrupted both or individual mTOR complexes in epidermis by conditional gene targeting. We found that mTOR signaling is essential for skin morphogenesis as epidermal-specific *Mtor* mutants ($mTOR^{EKO}$) are viable but die shortly after birth due to lack of a protective epidermal barrier. Interestingly, epidermis-specific loss of *Rptor* (Rap^{EKO}), which encodes an essential component of mTORC1, confers the same skin phenotype as seen in $mTOR^{EKO}$ mice. In contrast, newborns with an epidermal deficiency of *Rictor* (Ric^{EKO}), an essential component of mTORC2, survive despite a hypoplastic epidermis. Loss of epidermal mTORC1 activity attenuated basal cell growth and proliferation, which further led to abrogate the epidermal stratification and hair follicle formation. Ric^{EKO} mutants were characterized by a delayed epidermal maturation, displaying hypoplastic epidermis and increased transepidermal water loss. Mechanistically mTORC2 acts during epidermal stratification at least in part by regulating basal cell polarization and division orientation, which is critical for initiating the formation of a protective epidermal barrier. Collectively, our work highlights a fundamental role for mTOR signaling in epidermal morphogenesis and we reported a previously unknown signaling pathway that is required for the induction of epidermal stratification and barrier formation.

Perspectives

There is an emerging appreciation for the critical contribution of mTOR signaling in skin physiology and pathology. Our findings have now added novel and substantial insight to these processes. Ongoing studies in our group focus on the translation of our findings in mice into a better understanding of skin pathologies in human.

Mechanisms of stem cell apoptosis in epidermal regeneration

A. Geueke[1,2] / K. Reuter[1,2] / C. Niemann[1,2]

[1]Center for Molecular Medicine, University of Cologne, Germany
[2]Center for Biochemistry, University of Cologne, Germany

ageueke@uni-koeln.de

Self-renewing tissues like the mammalian epidermis and its appendages go through cycles of proliferation, terminal differentiation and apoptosis. To maintain tissue integrity and stem cell (SC) function, these diverse processes need to sustain constant environmental assaults, including UV radiation.

In the mammalian epidermis, multiple SC compartments have been identified, including SCs in the basal layer of the interfollicular epidermis and the hair follicle bulge. Bulge SC are the primary cellular source for hair follicle renewal and comprise at least two different subpopulations, basal and suprabasal bulge SCs. Previous data show, that bulge SCs are equipped with specific surveillance mechanisms protecting them against accumulation of DNA damage and unwanted cell loss to maintain tissue homeostasis.

Importantly protection of hair follicle SCs against DNA damage-induced apoptosis correlates with high levels of the anti-apoptotic protein Bcl-2. However, the specific function of Bcl-2 for bulge SCs, particularly its role in heterogeneous SC response and the relevance for SC survival and disease initiation, is not known. To address these important issues, we focussed on the following questions: 1. How is Bcl-2 involved in cell death regulation in heterogeneous hair follicle SC populations? and 2. What is the consequence of abnormal SC-apoptosis for epidermal tissue regeneration? Our results demonstrate that systemic Bcl-2 inhibition induces SC-specific cell death of mainly quiescent SCs and cause depletion of predominantly suprabasal bugle SCs. As a consequence subsequent renewal of the hair follicles is attenuated.

This data reveal physiological relevance of SC heterogeneity and suggest a novel, important role of suprabasal bulge SCs for hair follicle regeneration, depending on Bcl-2-mediated protection during SC quiescence.

Functional analysis of ***mthl7*** and ***mthl8*** in context of aging and stemness in the female germline stem cells of ***Drosophila melanogaster***

B. Giriyapura [1,2] / M. Tiwari [1,2] / A. Wodarz [1,2]

[1]Molecular Cell Biology, Institute of Anatomy, University of Cologne, Germany
[2]CECAD Cologne, Germany

bharath.giriyapura@uk-koeln.de

Introduction

The ovaries and testes of Drosophila melanogaster are attractive models to study the molecular basis of aging and stemness. These tissues contain Germline stem cells (GSCs) that divide to ultimately result in the production of functionally mature gametes. In wild type flies, GSC stemness declines with age, but male GSCs of the long-lived *methuselah* (*mth*) mutants exhibit persistent activity up to 35 days. However, the molecular relation of stemness to aging has not been studied and is still ambiguous in this system.

To uncover stemness factors in GSCs, we carried out RNA sequencing on GSC-like cells (mutant for *bag-of-marbles* (*bam*)) from Drosophila ovaries. Surprisingly, we found 5 members of the Mth family (*methuselah-like, mthl*) to be significantly differentially expressed. We hypothesize that Bam and Mthl7/8 might interact to affect aging and stemness in Drosophila GSCs.

We initially focus on two *mthl* genes, *mthl7* and *mthl8*, that are significantly upregulated in *bam* mutant GSC-like cells. We aim to functionally characterize these genes in context of aging and stemness, and study the molecular and phenotypic effects of bam mutation induced *mthl7/8* upregulation on female GSCs of Drosophila.

Results

The germline specific and somatic knockdown (kd) of 5 members of the *mth-like* genes shows decreased maximum mean lifespan as compare to control animals. Initially we screened these *mthl* genes with different RNAi lines to observe the GSC number with age using germline markers. We did not detect any significant change in the stem cell number with age and observed normal cogenesis process. To investigate whether *Mthl7/8* are functionally relevant *bam* target genes we aimed to rescue the bam loss of function phenotype by single and double kd of *mthl7* and *mthl8* in *bam* mutant background but did not observe any significant difference compare to *bam*-RNAi kd ovaries. Our preliminary results show that there is no genetic interaction between *Bam* and *Mthl7/8* to affect aging and stemness in Drosophila female GSCs.

Perspectives

It is known that, Methuselah (Mth) is a secretin-incretin receptor subfamily member required in the insulin-producing cells for proper nutrient coupling. Presently we study whether mth and insulin signal coupling impact on cell cycle of GSCs.

Cellular polarity and metabolic signalling in sebaceous gland homeostasis

K. Göbel[1] / C. Mann[2] / E. Wachsmuth[2] / S. Brodesser[1] / M. Schneider[3] / X. Lim[4] / M. van Steensel[4] / C. M. Niessen[2]

[1]University of Cologne, Cologne Excellence Cluster on Cellular Stress Responses in Aging-associated Diseases (CECAD), Cologne, Germany
[2]University of Cologne, Department of Dermatology, Cologne, Germany
[3]Ludwig-Maximilians-Universität Munich, Gene Center, Munich, Germany
[4]Institute of Medical Biology, Agency for Science, Technology and Research, Singapore

klaus.goebel@uni-koeln.de

Sebaceous glands secrete a complex lipid mixture known as sebum onto the skin surface, and are subject to constant cellular turnover. One of the most prevalent skin diseases in humans, acne vulgaris, leads to enlarged sebaceous follicles.

Whether this is due to a defective lipid metabolism, altered cellular turnover or improper sebaceous cell fate specification remains largely elusive. Here, we show that knockout of the cellular polarity factor aPKC in the Lrig1-positive sebocyte progenitor compartment does not result in enlarged sebaceous glands, but rather leads to a shift in cell fate towards differentiation in the interfollicular epidermis. Sebocyte lipid metabolism requires tight regulation, although the underlying mechanisms are not properly understood. Interestingly, acne patients have an increased Insulin-like Growth Factor (IGF)-1 serum concentration. Here we asked whether epidermal insulin and IGF-1 signaling (IIS) determines sebaceous gland formation, homeostasis and lipid production. Epidermal inactivation of the IGF-1 receptor (IGF-1R) results in smaller sebaceous glands in adult mice, although sebocyte cell size is not altered.

This is likely due to specification, as the sebocyte number is already impaired in newborn epidermal IGF-1R knockout mice and epidermal loss of IIS results in a complete loss of the sebocyte lineage. We next asked whether IIS directly controls sebocyte function. Inactivation of IIS only in sebocytes using SCD3-Cre mice had no obvious effect on gland morphology. At present we are inactivating IIS only in Lrig1-positive to directly ask whether IIS controls the sebaceous lineage. Together, our data suggest that IIS controls sebaceous gland development and maintenance through regulation of either sebocyte progenitor cell turnover or cell fate determination, rather than sebocyte metabolism, providing potential novel insights into the pathogenesis of acne vulgaris.

Plasticity, convergence and legacy of Lgr5 and Lgr6 stem cells during cutaneous wound repair

S. Joost[1] / T. Jacob[1] / X. Sun[1] / M. Kasper[1]

Center for Innovative Medicine & Department of Biosciences and Nutrition, Karolinska Institute, Stockholm, Sweden

tina.jacob@ki.se

The mouse epidermis with its hair follicles constitutes an excellent model system for studying tissue maintenance and repair. Using single-cell transcriptomics and lineage-tracing mouse models, we explore cellular heterogeneity during homeostasis and the adaptability of cells during wound repair. Previously, we investigated the epidermis at single-cell resolution to molecularly profile its full repertoire of cells, and described spatiotemporal gene expression patterns and the heterogeneity of stem and progenitor cells[1].

Here, we studied transcriptional changes of Lgr5 and Lgr6 hair follicle stem cells and their progeny while contributing to wound repair. We analyzed cells at the time of wounding, and 1 day, 4 days, 7 days, 10 days and 1 month after wounding. Single-cell transcriptomics analysis revealed six cell clusters representing distinct cellular states during the re-epithelialization process. The results show, that wound cell states describe the gradual changes during wound repair much better than the sampling time points. Importantly, the wound cell states allowed for direct comparison between Lgr5 and Lgr6 progeny while contributing to re-epithelialization, revealing that Lgr6 stem cells and their progeny reacted much faster to injury than Lgr5 cells.

Moreover, Lgr5 progeny lost their typical bulge signature within a few days, and took on an interfollicular epidermis identity. During the wound repair process, cells derived from the Lgr5 and Lgr6 stem cell pools transcriptionally converged at the wound front and diverged again thereafter. In summary, using single-cell RNA-seq we could capture the transcriptional changes of recruited wound-healing cells originating from distinct stem cell niches, shedding light on the cellular plasticity during tissue repair.

References

1. Joost S, Zeisel A, Jacob T, Sun X, La Manno G, Lönnerberg P, Linnarsson S, Kasper M. Single-Cell Transcriptomics Reveals that Differentiation and Spatial Signatures Shape Epidermal and Hair Follicle Heterogeneity. Cell Syst. 2016 Sep 28;3(3):221-237.e9.

Mitochondrial dysfunction in muscle stem cells impairs regeneration

S. Kimoloi[1] / O. R. Baris[1] / R. J. Wiesner[1, 2, 3]

[1]Center for Physiology and Pathophysiology, Institute of Vegetative Physiology, University of Cologne, Germany
[2]Center for Molecular Medicine Cologne, University of Cologne, Germany
[3]Cologne Excellence Cluster on Cellular Stress Responses in Aging-associated Diseases, University of Cologne, Germany

obaris@uni-koeln.de

Introduction

The adult skeletal muscle stem cells (MuSc) play an important role in the regeneration of lost mature myofibers. However, MuSc regenerative efficiency declines with aging. We hypothesized that mitochondrial dysfunction due to altered mitochondrial DNA (mtDNA) integrity might be one of the intrinsic factors in the aging-dependent decline of MuSc regenerative capacity. To test this, we generated a mouse strain which expresses a dominant-negative mutant of the mitochondrial helicase Twinkle (K320E-Twinkle^{MuSc}) upon Pax7-Cre-mediated recombination. Patients harboring mutations in the Twinkle gene accumulate multiple mtDNA deletions in various tissues. Thus, in K320E-Twinkle^{MuSc} mice, mtDNA integrity is compromised specifically in MuScs.

Results

One tibialis anterior (TA) muscle of these mice was injured by cardiotoxin at around 12 weeks of age, to stimulate the regeneration process. Mitochondrial dysfunction and regeneration efficiency were then analyzed at 7, 30 and 120 days post-injury by cytochrome c oxidase/succinate dehydrogenase activity staining and by hematoxylin/eosin staining respectively. The proportion of regenerated fibers with cytochrome c oxidase deficiency (COX deficient), a hallmark of cells with respiratory chain dysfunction driven by mtDNA alterations, was 31%, 17% and 14% at 7, 30 and 120 days post-injury, respectively. This was accompanied by time-dependent atrophy of the COX deficient fibers and increased fibrosis. The regenerated K320E-Twinkle^{MuSc} TA muscles also exhibited increased content in adipocytes and immune cells as well as delayed peripheral nuclear positioning. Consequently to this altered regeneration process, the mass of the regenerated muscles was lower in K320E-Twinkle^{MuSc} compared to control 30 days post-injury.

Perspectives

Collectively, these findings indicate that compromised mtDNA integrity in MuSc is detrimental for skeletal muscle regeneration. Thus, strategies aimed at maintaining this parameter in MuSc might potentially sustain the regenerative capacity of skeletal muscle during aging. The generated K320E-Twinkle^{MuSc} mice appear as a valuable model to test such strategies in the future, as well as investigating the mechanisms via which mitochondrial dysfunction in MuSc promotes muscle fibrosis and fat cell infiltration.

Epithelial stem cell fate plasticity: a mouse oesophageal model

J. McGinn[1] / P. Jimenez-Gomez[1] / M. P. Alcolea[1]

Wellcome Trust/Medical Research Council Cambridge Stem Cell Institute and Department of Oncology, University of Cambridge, UK

mpa28@cam.ac.uk

Epithelial tissues have evolved to respond to environmental challenges. They must react rapidly to tissue disruption in order to restore the epithelial barrier and ensure survival of the organism. Dysregulation of this process would have devastating consequences, leading to epithelial disease and cancer.

It has traditionally been thought that most tissues rely on a defined stem cell subpopulation for tissue maintenance and repair, presenting tumour initiating potential when acquiring the relevant mutations. However, mounting lines of evidence suggest that cell fate is highly dynamic.

Following injury and tumorigenesis, differentiating or lineage committed cells may reacquire regenerative potential and stem cell-like behaviour, providing some clues as to why cancer stem cells have remained controversial so far.

Using the mouse oesophagus as a model, and by combining genetic lineage tracing approaches with methods from statistical physics, we have revealed the remarkable cellular plasticity of epithelial cells in this tissue. Cell are not only able to redefine the programme of cell behaviour in response to injury and early tumour formation, but they are also able adapt to perturbations by reaching new steady states contributing to maintain tissue integrity.

New insights on oesophageal post-natal development show how during the first two weeks after birth progenitor cells rapidly adapt and change their behaviour to support the remarkable growth of the tissue and its new digestive function.

This fast expanding mode is then lost leading to the onset of homeostasis. We propose that this plasticity is a mechanism hijacked by tumour forming cells to evolve during disease progression and resistance.

Polarity signaling in the regulation of spindle orientation

J. Nafisi[1,2] / F. Tellkamp[1] / S. Vorhagen[1] / C. M. Niessen[1]

[1]Department of Dermatology, Cluster of Excellence in Cellular Stress Responses in Aging-Associated Diseases (CECAD), Center for Molecular Medicine Cologne (CMMC), University of Cologne, Germany
[2]Cologne Graduate School for Ageing Research, University of Cologne, Germany

jnafisi@smail.uni-koeln.de

Adult Stem cells are crucial regulators of tissue homeostasis by tightly balancing self-renewal and differentiation. Perturbing this balance results either in overgrowth and cancer or in a decline in regenerative potential of tissues. However, how stem cells maintain this balance is only poorly understood. One key process by which stem cells are thought to maintain tissue homeostasis is through oriented cell division in which cells couple spindle positioning to the localization of extrinsic and/or intrinsic cell fate determinants to two daughters either with equal fate (symmetric) or with differential fate (asymmetric).

Studies from lower organisms identified the polarity protein atypical protein kinase C (aPKC) as a key regulator of oriented cell division. Mammals have two aPKC isoforms, aPKCl and aPKCz. Our laboratory showed that epidermal loss of aPKCl promotes perpendicular spindles accompanied by stem cell loss, cell fate changes and premature skin aging, suggesting that aPKCl regulates cell fate through oriented cell division. Surprisingly, epidermal expression of a constitutve active aPKC also promoted perpendicular spindle orientation but resulted in an expansion of stem cells, suggesting that spindle orientation does not determine cell fate or these processes are uncoupled in the latter. To address how aPKC controls spindle orientation and whether this is linked to cell fate regulation, we first inactivated both mammalian aPKCs in the epidermis.

Surprisingly, this resulted only in loss of planar spindles but not perpendicular, as expected based on results from lower organisms. Proteomic analysis revealed that aPKC regulates potential cell fate determinants such as Lgl as well as spindle orientation machinery proteins, such as NuMA. To examine how aPKC affects the spindle orientation machinery we have established an in vitro live cell imaging assay for keratinocytes expressing NuMA-GFP under the K14 promoter. Inhibiting aPKC in these cells resulted in transient appearance of NuMA clusters during mitosis. Furthermore, as observed in vivo, loss of aPKC isoforms in cell culture resulted in a shift towards more perpendicular spindles.

Using this assay we now examine how regulators of spindle machinery such as NuMA and LGN affect spindle orientation downstream of aPKC and ask whether these changes in spindle orientation affect the localization of cell fate regulators, such as Lgl and Numb.

Pre-activated murine bone marrow-derived mesenchymal stem cells drive M2b polarization

D. Philipp[1] / Y. H. Choi[1] / T. Wahlers[1] / A. Paunel-Görgülü[1]

Department of Cardiothoracic Surgery, Heart Center of the University of Cologne, Germany

denise.philipp@uk-koeln.de

Introduction

In recent years it has become clear that mesenchymal stem cells (MSCs) do not only have the ability to differentiate into different cell types but also possess immunomodulatory properties. However, the underlying mechanism of MSCs-mediated immunoregulation is not fully understood so far. Macrophages are distinguished in classical activated, proinflammatory M1 and anti-inflammatory M2 cells, which possess different functions and transcriptional profiles with respect to inflammatory responses. In this study, we have explored the immunosuppressive effects of bone marrow-derived MSCs in respect to their ability to promote a shift in macrophage phenotype in dependence of their activation state. For this, murine bone-marrow derived MSCs were co-cultured with macrophages under M1and M2 polarizing conditions.

In some experiments, MSCs were pre-activated by interferon gamma (IFN-ɣ) und interleukin-1 beta (IL-1ß). Both M1 macrophages as well as three different M2 macrophage subtypes (M2a, M2b and M2c) were further characterized by real time PCR, flow cytometry and western blot. We hypothesize that pre-activation of MSCs might improve their immunosuppressive actions and might therefore represent a better strategy for their future application in clinic.

Results

Pre-activation of MSCs resulted in a strong increase *of inducible nitric oxide* (*iNOS)* expression accompanied by increased amounts of secreted NO and proinflammatory IL-6 in the culture supernatants. Co-culture experiments under M1 polarizing conditions revealed a significant downregulation of CD86, intracellular iNOS expression and secretion of pro-inflammatory tumor necrosis factor alpha (TNF-α) in macrophages in the presence of pre-stimulated MSCs independent of direct cell contact. Additionally, under M2-polarizing conditions, macrophages were found to upregulate CD86, iNOS and IL-10 indicating M2b polarization in the presence of activated, but not in the presence of unstimulated MSCs.

Indeed, MSC-mediated polarization toward a M2b phenotype in M1- as well M2-like cells was confirmed by strong upregulation of the M2b markers *sphingosine kinase (SPHK1)* and *LIGHT*, whereas only weak changes in M2a-specific *chitin-like 3 (Ym1)* and M2c-typical *Mer tyrosine Kinase (MertK)* could be observed.

Perspectives

Pre-activated MSCs supress M1 polarization and rather promote a polarization shift towards an anti-inflammatory M2b phenotype which is independent on direct cell cell contact. The identity of the soluble factors involved in MSCs-dependent immunoregulation is still elusive and needs further investigation in the future.

E-cadherin controls EGFR signaling and tissue tension for polarized epidermal junction and barrier formation

M. Rübsam[1,2,3] / A. Mertz[4] / A. Kubo[5] / E. Dufresne[4], V. Horsley[4] / W. Ziegler[6] / M. Amagai[5] / S. Wickström[7] / C. Niessen[1,2,3]

[1]Department of Dermatology, University of Cologne, Germany
[2]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD)
[3]Center for Molecular Medicine Cologne, University of Cologne, Germany
[4]Yale University, New Haven, USA
[5]Department of Dermatology, Keio University School of Medicine, Tokyo, Japan
[6]Hannover Medical School, Hannover, Germany
[7]Paul Gerson Unna Group 'Skin Homeostasis and Ageing, Max Planck Institute for Biology of Ageing, Cologne, Germany

ruebsam0@uni-koeln.de

Generation of a barrier in multi-layered epithelia like the epidermis requires restricted positioning of functional tight junctions (TJ) to the most suprabasal viable layer. This positioning necessitates tissue-level polarization of junctions and the cytoskeleton through unknown mechanisms. Previously, we showed that epidermal E-cadherin is essential for epidermal TJ barrier function. Using quantitative whole-mount imaging, genetic ablation, and traction force microscopy, we find that ubiquitously localized E-cadherin coordinates tissue polarization of vinculin positive, tension-bearing adherens junction (AJ) and F-actin organization and allows formation of an apical ZO1-EGFR-positive TJ network only in the granular layer 2 (SG2).

The SG2 layer itself is highly polarized despite its flattened appearance with a lateral AJ network reaching up to a continuous ZO-1 positive apical tight junctional ring. We find that E-cadherin regulates spatiotemporal positioning of EGFR receptor during epidermal barrier development at embryonic day 16.5 and balances non-mitogenic EGFR activity critical for in vitro TJ barrier function. Our data suggest that actomyosin mediated tension at AJ is required to tune EGFR activity levels allowing spatially restricted stabilization of TJ complexes in the SG2. In turn, we find EGFR activity itself to feed back into regulating actomyosin contractility.

In conclusion, our data link E-cadherin-dependent control of suprabasal EGFR activity and the polarized, tissue-level organization of junctions, tension, and the cytoskeleton to promote in vivo epidermal barrier formation and tissue turnover. Our results further reveal a mechanistic role for EGFR at TJs and thereby uncover why EGFR inhibitors compromise skin barrier function in human cancer patients.

A Myc-Tip60 transcriptional network maintains ***Drosophila*** neural stem cells by regulating the polarity determinant aPKC

K. Rust[1,2,3] / M. Tiwari[1,2,3] / A. Wodarz[1,2,3]

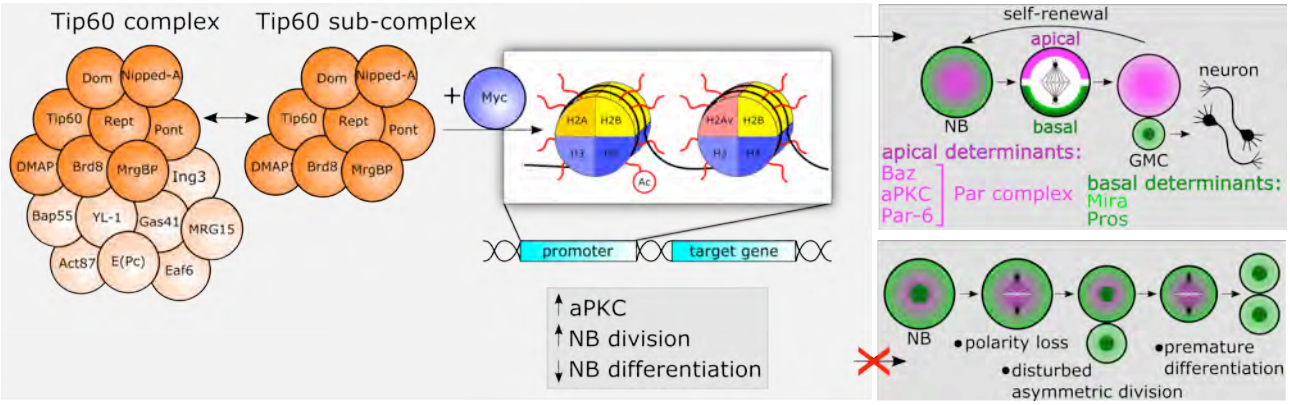
[1]Molecular Cell Biology, Institute I for Anatomy, University Medical Center Cologne, Germany
[2]Cluster of Excellence - Cellular stress response in aging-associated diseases (CECAD), Cologne, Germany
[3]Stem Cell Biology, Institute for Anatomy and Cell Biology, Georg-August University Göttingen, Germany

krust@uni-koeln.de

Stem cells establish cortical polarity and divide asymmetrically to simultaneously maintain themselves and generate differentiating offspring cells. Several chromatin modifiers have been identified as stemness factors in mammalian pluripotent stem cells, but how they control stem cell polarity and asymmetric division is unknown.

We addressed this question in *Drosophila* neural stem cells called neuroblasts. In a comprehensive reverse genetic screen of chromatin modifiers, we identified a Tip60 chromatin remodeling sub-complex which interacts with the transcription factor Myc in neuroblast maintenance. Knockdown of members of this network results in symmetric neuroblast division, loss of cortical polarity and premature differentiation through nuclear entry of the transcription factor Prospero.

The Tip60 complex regulates gene expression by histone acetylation of incorporation of H2A variants. Using transcriptome analysis we identified the polarity regulator and Par complex member aPKC as a key target gene of the Tip60 sub-complex and Myc. Overexpression of aPKC rescues some but not all aspects of the Tip60 and Myc knockdown phenotype. Our findings reveal a functional link between evolutionarily conserved stemness factors and the molecular network controlling cell polarity.



In order to identify the direct targets of the Myc-Tip60 network we plan to apply the DamID method on FAC-sorted neuroblasts. Identifying targets regulating the maintenance of *Drosophila* stem cells will also provide a potential starting point for studies on conserved stem cell factors in mammals and can thus contribute to our understanding of stem cell regulation.

Embryonic loss of HCN/h current impedes development of the cerebral cortex by reducing neural stem cell proliferation

A.K. Schlusche[1,2] / S. Vay[3] / Adele Rüger[3,4] / Dirk Isbrandt[1,2,4] / I. Jakovcevski[1,2,4]

[1]Institute for Molecular and Behavioral Neuroscience, University of Cologne, Germany
[2]Experimental Neurophysiology, German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany
[3]Department of Neurology, University Hospital of Cologne, Germany
[4]Center for Molecular Medicine Cologne, Germany

igor.jakovcevski@uk-koeln.de

The development of the cerebral cortex is a complex process comprising cell division, differentiation, migration, axonal pathfinding, and synaptogenesis. For all processes involved in cortical development the spontaneous activity including calcium waves in neurons and their precursor cells, mediated by intrinsic and extrinsic properties, is of great importance. The extrinsic properties mainly arise from stimulation of neurotransmitter receptors via synaptic or volume transmission, whereas the intrinsic biophysical properties of precursor cells and neurons are defined by their ion channel composition. The hyperpolarization-activated cyclic nucleotide-gated non-selective cation (HCN) channels mediating the h-current (I_h) shape the biophysical properties of neurons throughout brain development. HCN channels consist of four subunits that assemble into homo- or heteromeric tetramers. We generated a transgenic mouse line expressing a dominant-negative HCN subunit (HCN-DN) that led to the functional suppression of I_h independent of the endogenous subunit composition. By expressing the transgene under control of the EMX1 promoter, I_h suppression starts at embryonal day (E) 9.5 in a forebrain-restricted manner.

The functional ablation of I_h in early prenatal brain development resulted in a severe phenotype with pronounced microcephalus and reduced neonatal viability, with reduced cell proliferation at postnatal day 0. *In utero* electroporation of HCN-DN into the lateral ventricle performed at E15 and analyzed at E19 suggests no alteration in neuronal migration. To assess the impact of I_h blockade on differentiation *in vitro*, we blocked I_h using ZD7288 in rat cortical neural stem cells.

These results show decreased proliferation and increased glial differentiation of stem cells upon I_h blockage. Our data support the hypothesis that I_h is an important intrinsic regulator of the proliferation and differentiation of neural progenitors, and that mutations in HCN channel genes could lead to severe brain malformations.

From murine induced pluripotent stem cells (iPSCs) to vascular wall-typical mesenchymal stem cells (VW-MSCs) – using a VW-MSC specific Gene-code

J. Steens[1] / M. Zuk[2] / N. Teichweyde[2] / J. Hess[3] / K. Unger[3] / H. Klump[2] / D. Klein[1]

[1]Institute of Cell Biology (Cancer Research), University Hospital Essen, Medical Faculty, University of Duisburg-Essen, Essen, Germany
[2]Institute for Transfusion Medicine, University Hospital Essen, University of Duisburg-Essen, Essen, Germany
[3]Research Unit Radiation Cytogenetics, Helmholtz Zentrum Munich, Neuherberg, Germany

jennifer.steens@uk-essen.de

Introduction

Bone marrow is a frequently used source of mesenchymal stem cells (MSCs). Such MSCs are used for the treatment of steroid-refractory graft-versus-host disease after transplantation of hematopoietic stem cells because of their weak allogenic immune response. However, bone marrow extraction is a highly invasive procedure and only 0.01 % to 0.001 % of the collected cells are MSCs. Therefore, more easily accessible sources of MSCs are needed.

MSCs can also be isolated from various other adulte human tissues e.g. cord blood, placenta, peripheral blood, adipose tissue, synovia, lung, fetal liver and from the vessel wall (VW). But the variations of the qualtiy of obtained donors and tissues sources as well as subseqent cell culture, have led to numerous inconsistencies in the reported in vivo effectiveness of MSCs. An alternative method to avoid these issues is the generation of MSCs from induced pluripotent stem cells (iPSCs) *in vitro*. Here we report the in vitro generation of VW-typical MSCs from induced pluripotent stem cells (iPSCs), based on a VW-MSCspecific gene code.

Results

Dermal tail fibroblasts isolated from transgenic mice containing GFP gene integrated into the Nestin-locus (NEST-iPSCs) to facilitate lineage tracin after MSC differentiation were reprogrammed to iPSCs by using a lentiviral vector expressing the so-called Yamanaka factors Oct3/4, Sox2, Klf4 and c-Myc. A lentiviral vector expressing a small set of recently identified human VW-MSC-specific HOX genes then induced MSC differentiation.

This direct programming by using the three VW-MSC-specific HOX-Genes B7, C6 and C8 successfully mediated the generation of VW-typical MSCs with classical MSC characteristics, both in vitro and in vivo. In vitro, the MSCs adhered to plastic and were able to differentiate along different mesodermal lineages providing evidence for their multipotency and MSC-like differentiation potential. *In vivo*, the VW-MSCs were able to form teratomas. They were found to be associated with newly formed blood vessels, which nicely correlated with the observed increased size of the teratomas grown from HOX-transduced NEST-iPSCs.

Perspectives

The possibility and feasibility of obtaining patient-specific VW-MSCs from iPSCs in large amounts by forward programming could potentially open avenues toward novel, MSC-based therapies. As the activity of homeotic selector proteins are highly conserved throughout evolution, it is very likely that our results will also hold true for human iPSCs. These cells then may be particularly well suited for the treatment of diseases associated with vascular damage and remodeling, such as hypertension, ischemic diseases, congenital vascular lesions, shear stress, or irradiation.

Analysis of Dermal Stem Cells in Healthy and Diseased Skin

B. Széky

Stem Cell Research Laboratory, Semmelweis University,
Department of Dermatology, Venereology and Dermatooncology, Budapest, Hungary

szekyb@gmail.com

The mammalian dermis is a source of heterogeneous fibroblastic cells with different origin and versatile roles in tissue homeostasis. In the past few years, special subpopulations identified among the diverse subsets of fibroblasts with stem cell properties and advanced immunomodulatory potential. Some of them are even regarded as adult pluripotent stem cells participating in wound healing, reepithelization and *de novo* hair follicle morphogenesis. Despite their regenerative capacities being extensively studied *in vivo*, their origin, clonal relationships, distinguishing markers and differentiation potential are not comprehensively established.

Here I present data on methods to isolate multilineage differentiating, stress enduring (MUSE) cells, skin derived precursors (SKPs) and mesenchymal stem/stromal cells (MSC) from human dermal samples including the optimalization their enrichment in cell culture and *in vitro* differentiation. I will also present data how the different stem cell markers differ or overlap between MUSE cells, SKPs and dermal MSCs and whether interconversion exists among these stem cell subsets. These experiments are important to address the function of these stem cells not only in healthy tissues, but under the influence of a tumor microenvironment to understand how melanomas exploit dermal stem cells to support their growth and propagation.

Disturbed metabolic regulation of skin barrier function in hyperglycemic and type 2 Diabetes

E. Wachsmuth[1] / M. Awazawa[3] / S. Y. Aghdam[1] / F. Tellkamp[1] / M. Pasparakis[2] / J. C. Brüning[3] / C. M. Niessen[1]

[1]University of Cologne, Department of Dermatology, Cologne Germany
[2]Cologne Excellence Cluster on Cellular Stress Responses in Aging-associated Diseases (CECAD), Cologne,Germany
[3]University of Cologne, Center for Endocrinology, Diabetes and Preventive Medicine, Cologne, Germany

ewachsm1@uni-koeln.de

Type 2 diabetes mellitus is increasingly prevalent in the western population and is associated with a plethora of skin diseases, such as impaired wound healing and increased infections. Yet, it remains unresolved whether diabetic skin complications result secondarily in response to metabolic alterations or from impaired insulin action in the skin or other organs.

A major aim is thus to define the contribution of cell autonomous and non-cell autonomous insulin/IGF signalling (IIS) in skin barrier function and in development of diabetes-associated skin complications. We recently showed that epidermal Insulin receptor (IR) and Insulin-Like Growth Factor receptor (IGF-1R) signaling (IIS) controls p63 dependent stratification early during epidermal morphogenesis. The functional epidermal barrier in mice is first formed at embryonic Day (E) 16.5 and protects the organism of chemical and physical insults and serves as immune barrier. We now find that loss of epidermal IIS signaling results in an initially severe barrier defect at E17.5, however this defect is much less pronounced in newborn mice, thus suggesting a compensatory response. On the molecular level, loss of IIS induces p38a Map kinase stress response that promotes epidermal barrier formation as combined loss of IIS and p38 in the epidermis resulted in severe barrier defects and perinatal lethality. Spike-in Silac on newborn epidermis identified the Epidermal Differentiation complex as one key target of p38 and IIS signaling.

Together, the data show that activation of p38 signaling partially repairs the stratum corneum barrier defects induced by reduced epidermal IIS signaling. Our data also provide direct evidence that epidermal impaired IIS alters skin barrier function. Importantly, we find that adult dietary and genetically obese mice present with insulin resistance in skin, exhibit barrier dysfunction and epidermal atrophy, thus linking impaired cell autonomous IIS in the epidermis to diabetic associated skin complications.

Notes

Role of endogenous bioelectric currents in controlling cell differentiation

M. Bhavsar / J. H. Barker

Experimental Orthopedics and Trauma Surgery, J.W. Goethe University Frankfurt, Frankfurt, Germany

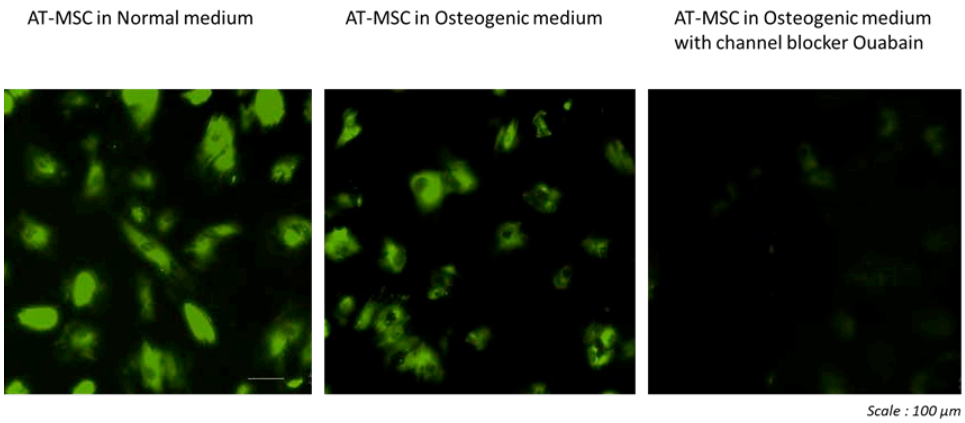
mbhavsa@gwdg.de

Introduction

Harnessing the potential of stem cells for bone healing and tissue regeneration is exciting but still a challenging task. Cell proliferation, migration, differentiation and adhesion are critical for forming biologically and structurally viable compounds for repair of damaged tissue. While there is a limited data available about the electrophysiological role of stem cells, slow changes in steady state transmembrane potentials (Vmem) of the plasma membrane driven by ion channels, pumps and gap junctions are known to be involved in bioelectric signaling and thus controlling regeneration[1,2]. Probing the function of such bioelectric gradients in-vivo or in culture requires to track the Vmem over the period of cell proliferation, migration and differentiation.

Results

To provide the basis of membrane potential changes we used voltage sensitive fluorescent dye bis-(1,3-dibutylbarbituric acid)-trimethine Oxonol (DiBAC4(3)) and followed changes in the membrane potential of Adipose tissue derived mesenchymal stem cells (AT-MSC) undergoing osteogenic differentiation (day 0, day7, day 14 and day 21) and found that Vmem is exponentially decreasing over the period of differentiation which suggests that Vmem is functionally determinant of AT-MSC differentiation. To find out if Vmem plays any role in controlling the cell differentiation process, we tried reversal of progressive differentiation using channel blocker Ouabain (modulation of Vmem) showed reduction in on-going differentiation. Collectively, these data suggest that Vmem is one of the parameter that controls osteogenic differentiation of AT-MSC.



Perspectives

Controlling bioelectrical properties of stem cells is not only a fascinating but also useful tool in regenerative medicine. Regulating Vmem using any external modality (ie direct current electricity) will be potentially useful for rational modulation of stem cell function.

References

1. Sundelacruz S, Levin M, Kaplan DL. Membrane potential controls adipogenic and osteogenic differentiation of mesenchymal stem cells. PLoS One. 2008;3(11). doi:10.1371/journal.pone.0003737.
2. Sundelacruz S, Levin M, Kaplan DL. Role of membrane potential in the regulation of cell proliferation and differentiation. Stem Cell Rev Reports. 2009;5(3):231-246. doi:10.1007/s12015-009-9080-2.

Possible mechanisms of peptic ulcer non-healing after bleeding

V. Delii[1] / S. Zharikov[2] / P. Kondratenko[2] / O. Sulaieva[1]

[1]Department of Pathology, Ukrainian research and practical center of endocrine surgery, Ukraine
[2]Department of Surgery, M. Gorky Donetsk National Medical University, Ukraine

oksana.sulaieva@gmail.com

Introduction

One of the most often causes of upper gastro-intestinal bleeding is peptic ulcers. Despite the progress in endoscopic hemostasis and development of gastrointestinal protection strategy it is still high rate of recurrent ulcer bleeding. Previously it was shown that lack of ulcer repair is due to inflammatory reaction leading to alteration of healing and unsustainable hemostasis. Despite the fact, that platelets are considered as an important moderator of ulcer healing, there are just few data on relationship between platelets function, inflammatory reaction and ulcer healing after bleeding. The aim of this study was to assess the interplay between platelets' activity, inflammation and ulcer healing among patients with gastrointestinal ulcer bleeding.

Methods

70 patients (males, 52±3 years old) with peptic ulcers complicated with acute gastrointestinal bleeding (Forrest II class) were enrolled in the study. According to the outcome of UB all patients were subdivided into two groups – with sustained hemostasis (1st group) and rebleeding (2nd group). All the enrolled patients provided informed consent. Platelets' activity was estimated by aggregation test, induced by ADP (5 μM) and collagen (1 μM). Inflammatory reaction was assessed histologically by evaluation of vascular reaction, oedema damage, infiltration with neutrophils, lymphocytes and macrophages (CD68) number. To assess ulcer healing we focused on granulation tissues formation and epithelization, counting myofibroblasts (α-SMA), CD31 and Ki-67 positive cells in and biopsies of gastric or duodenal mucosa at ulcer margin. Biopsy was done within 24 hours of UB symptoms onset.

Results

It was shown that acute ulcer bleeding is associated with decrease of collagen- and ADP-induced aggregation of platelets (p=0,01). However, the value of platelets aggregation was not associated with bleeding outcome. Rebleeding development in 2nd group patients was associated with reversible type of platelets aggregation curve. Such kind of curve can reflect the alteration of degranulation mechanisms and lack of growth factors release. These changes were accompanied with high intensity of acute inflammatory infiltration. The number of polymorphonuclear leukocytes (p<0.01) and macrophages (p<0,001) was significantly higher in 2nd group patients that was associated with prominent damage, edema and lack of repair. We found the decline of myofibroblasts number (p<0,001) among rebleeders, reflecting the alteration of myofibroblasts formation and/or proliferation. But there were no differences in Ki-67 positive cells with regard to bleeding outcome.
Thus, development of ulcer rebleeding is associated with exaggerated inflammatory reaction and platelets dysfunction with reversible aggregation pattern. These changes could be caused by alteration of degranulation and lead to un-sustained hemostasis, violation of ulcer healing and rebleeding development.

Perspectives

Further evaluation of platelet-leukocytes interplay after bleeding is essential for understanding and control of inflammation-induced damage of gastrointestinal duodenal mucosa and facilitation of its healing.

The RNA methyltransferase Nop2 is a novel regulator of liver regeneration

A. Fearon / T. Speicher / U. auf dem Keller / S. Werner

Institute of Molecular Health Sciences, ETH Zürich, Zurich, Switzerland

abbie.fearon@biol.ethz.ch

The liver is the only organ in mammals that can fully regenerate after injury. Hepatic tissue loss initiates a well-defined repair program, resulting in restoration of the initial liver mass and re-establishment of the liver's essential functions in metabolic regulation and compound detoxification. Although the regeneration process is well described at the histological level, only a few of the genes/proteins involved have been identified and functionally characterised.

Partial hepatectomy (PH), comprising surgical removal of 70% of the liver tissue, provides a suitable model to study liver regeneration in rodents. To delineate the molecular mechanisms responsible for liver regeneration following PH, protein levels of isolated nuclei and cytoplasm of wild-type mice were analysed using mass spectrometry at various time points post-PH. Interestingly, Nucleolar protein 2 (Nop2), a poorly characterised putative rRNA methyltransferase belonging to the Nsun family of proteins, was up-regulated in the nucleus 24 hours after PH.

The functional role of Nop2 in both the uninjured and regenerating liver was assessed *in vivo* using nanoparticle-mediated siRNA knock-down. Our data showed that, whilst hepatocyte proliferation was mildly decreased upon Nop2 knockdown under homeostatic conditions, liver function and survival of animals was unimpaired. However, knock-down of Nop2 significantly reduced the proliferative capabilities of hepatocytes and increased necrosis in the early phases of the regeneration process.

Expression of a range of cell cycle regulators and transcription factors crucial for liver regeneration was also significantly decreased. Further analysis showed that, whilst fewer animals survived five days post PH, those that did survive appeared to have regained their regenerative capabilities despite the lack of Nop2 expression. This may be due to a compensatory effect of other Nsun proteins as suggested by a significant increase in expression of a related rRNA methyltransferase, Nsun5, in primary hepatocytes after Nop2 siRNA knockdown.

We now aim to elucidate the mechanism by which Nop2 effects proliferation and cell survival in the regenerating liver via identification of its methylation targets and investigation of its role in ribosome biogenesis. Identification of the function of Nop2 and other Nsun proteins in liver regeneration will provide insight into the role of rRNA modifications and the composition of ribosomes in liver regeneration.

Treatment with 4-Methylumbelliferyl-β-D-xyloside and uridine diphosphate leads to an increased intracellular XT-activity in normal human dermal fibroblasts

B. Fischer / D. Hendig / J. Kuhn / C. Knabbe / I. Faust

Institute for Laboratory- and Transfusion Medicine, Heart and Diabetes Center NRW, Ruhr-University Bochum, Bad Oeynhausen, Germany

bfischer@hdz-nrw.de

Introduction

A fibrosis is defined as an excessive, abnormal deposition of extracellular matrix (ECM) molecules, which can affect different organs (e.g. skin, heart and the lung). These molecules are secreted by specific cells (e.g. fibroblasts), to maintain structural and biochemical support to surrounding cells. Although there are a lot of studies concerning the effect and inhibition of pro-fibrotic mediators, currently no appropriate therapy is available to treat the disease. Xylosyltransferases (XT) catalyze the rate-limiting step in proteoglycan biosynthesis by transferring activated xylose of UDP-xylose to a core-protein. Previous studies have shown, that an increase in *XYLT* mRNA expression as well as serum XT-activity is associated with diseases being characterized by an abnormal extracellular matrix remodeling as for instance fibrosis. Developing anti-fibrotic therapeutic strategies requires a broad knowledge of XT-specific transcriptional regulation processes. The aim of this study was to find molecules, which specifically inhibit XT-activity in normal human dermal fibroblasts (NHDF). Additionally, we examined the influence of these molecules on gene expression of different ECM molecules. For this purpose, we tested molecules of two different molecular classes (xylosides and nucleotides). 4-Methylumbelliferyl-β-D-Xyloside (MU-Xyl) has been shown to inhibit proteoglycan biosynthesis in different cell types. Furthermore, we tested the impact of the nucleotide Uridine diphosphate (UDP) on the expression of different genes of the ECM as well as on the XT-activity of NHDF cells.

Results

After treatment with 1 mM MU-Xyl, reduced mRNA expression levels of the genes *XYLT1*, smooth muscle alpha-2 actin (*ACTA2)*, elastin (*ELN),*aggrecan *(ACAN),* fibronectin *(FN1)* and beta-1,4-galactosyltransferase 7 (*B4GalT7)* were observed in treated cells. As opposed to this, *XYLT2* and collagen type 1 alpha 1 chain (*Col1A1)* mRNA expression levels were not significantly different compared to controls. The UDP-treatment (1 mg/ml) resulted in reduced mRNA expression levels of the genes *XYLT2, ACTA2, ELN, ACAN, Col1A1, FN1* and *B4GalT7*. There was no significant change in mRNA expression level of the *XYLT1* gene. Compared to controls, the relative intracellular XT-activity was significantly increased 72 h after treatment with both, MU-Xyl and UDP. 48 h after UDP-treatment, the extracellular XT-activity was decreased. As opposed to this, the extracellular XT-activity increased 72 h after treatment with UDP. With regard to MU-Xyl treatment, there were no significant changes in relative extracellular XT-activity for both time points.

Perspectives

Treatment with both molecules, MU-Xyl and UDP, leads to decreased mRNA expression levels of different ECM molecules in NHDF cells. Additionally, the treatments result in increased intracellular XT-activities after 72 h. The similar effects, initiated by the molecules, could be explained due to a regulation by the same signalling pathways. As a first approach, we determined the gene expression levels of different enzymes of the MAP-kinase (MAPK) pathway. With regard to this, we observed a downregulation of the Mitogen-activated protein kinase 3 (*MAPK3*) gene expression. In future studies, we would like to determine the underlying mechanisms induced by these molecules in more detail. This could additionally lead to important findings with regard to find a promising anti-fibrotic therapy.

Supported by the German Research Foundation (DFG).

Fibroblast heterogeneity in wound healing and fibrosis

S. Frech[1] / A. Korosec[1] / M. Mastrogiannaki[2] / F. M. Watt[2] / B. M. Lichtenberger[1]

[1]Skin & Endothelium Research Division (SERD), Department of Dermatology, Medical University of Vienna, Austria
[2]Centre for Stem Cells and Regenerative Medicine, Faculty of Life Sciences & Medicine, King's College London, UK

beate.lichtenberger@meduniwien.ac.at

Introduction

Dermal fibroblasts arise from two distinct lineages during embryogenesis and have unique functions in skin development and homeostasis. In mouse skin, one lineage forms the papillary fibroblasts located within the upper dermis including dermal papilla (DP) and dermal sheath (DS) cells that are required for the formation of the hair follicle and the arrector pili muscle. The other lineage forms the fibroblasts of the lower dermis including the reticular fibroblasts that synthesize the bulk of fibrillar extracellular matrix (ECM) as well as the preadipocytes and adipocytes of the hypodermis. Importantly, fibroblasts from the two lineages display differential expression of Wnt/β-catenin, Hedgehog and TGFβ pathway components and respond to different signals derived from neoplastic epidermal cells, thus remodelling the dermis with unique responses. Several lines of evidence suggest that also human skin comprises at least two fibroblast lineages with distinct functions. Interestingly, as skin ages, the gene signature and properties of fibroblasts changes, and papillary fibroblasts diminish in number leading to a progressive thinning of the skin.

Results

Using *in vivo* lineage tracing techniques, we show that the two fibroblast lineages also play distinct roles in regeneration and fibrosis. While reticular fibroblasts representing the majority of fibroblasts in adult tissue are important for the first phase of wound healing, the papillary lineage is not repopulated until re-epithelialization and contributes exclusively to the upper dermis, which explains, at least in part, the absence of hair follicles in newly closed wounds and scarring. Importantly, increasing the number of papillary fibroblasts prior to wounding alters the healing process and leads to *de novo* hair follicle formation within the wound bed. Similar to wound repair, also fibrosis is dominated by the reticular fibroblast lineage. We demonstrate that stabilizing Wnt/β-catenin signalling in reticular fibroblasts causes fibrotic lesions by preventing adipocyte differentiation and inducing proliferation, while papillary firbroblasts are not affected.

Perspectives

Our findings hold promise that modulating the abundance of papillary fibroblasts or dermal signalling can improve wound healing also in humans and eventually induce hair follicle neogenesis even in adult tissue, which could be pivotal for next-generation skin replacement therapy. In addition, targeting reticular fibroblasts or the signalling pathways deregulated in fibrotic cells may serve as new therapeutic approaches for patients suffering from fibrotic skin pathologies.

GATA4-dependent organ-specific endothelial differentiation controls liver development and embryonic hematopoiesis

C. Géraud[1]* / P.-S. Koch[1]* / J. Zierow[1]* / K. Klapproth[2] / K. Busch[2] / V. Olsavszky[1] / T. Leibing[1] / A. Demory[1] / F. Ulbrich[1] / M. Diett[1] / S. Singh[1] / C. Sticht[3] / K. Breitkopf-Heinlein[4] / K. Richter[5] / S.-M. Karppinen[6] / T. Pihlajaniemi[6] / B. Arnold[7] / H.-R. Rodewald[2] / H. G. Augustin[8] / K. Schledzewski[1] / S. Goerdt[1]

*These authors contributed equally to this work.

[1]Dept. of Dermatology, Venereology and Allergology, Univ. Medical Center and Medical Faculty Mannheim, Heidelberg University, and Center of Excellence in Dermatology, Mannheim, Germany
[2]Division of Cellular Immunology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[3]Center for Medical Research, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany
[4]Dept. of Medicine II, Section Molecular Hepatology, Medical Faculty Mannheim, Heidelberg Univ., Mannheim, Germany
[5]Division of Molecular Genetics, German Cancer Research Center (DKFZ), Heidelberg, Germany
[6]Faculty of Biochemistry and Molecular Medicine and Biocenter Oulu, University of Oulu, Oulu, Finland
[7]Division of Molecular Immunology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[8]Division of Vascular Oncology and Metastasis (DKFZ-ZMBH Alliance), German Cancer Research Center, Heidelberg, Germany, and Dept. of Vascular Biology and Tumor Angiogenesis (CBTM), Medical Faculty Mannheim, Heidelberg Univ., Mannheim, Germany

Johanna.Zierow@medma.uni-heidelberg.de

Microvascular endothelial cells (ECs) are increasingly recognized as organ-specific gatekeepers of their microenvironment. Microvascular ECs instruct neighboring cells in their organ-specific vascular niches through angiocrine factors, which include secreted growth factors (angiokines), extracellular matrix molecules, and transmembrane proteins. However, the molecular regulators that drive organ-specific microvascular transcriptional programs and thereby regulate angiodiversity are largely elusive. In contrast to other ECs, which form a continuous cell layer, liver sinusoidal ECs (LSECs) constitute discontinuous, permeable micro-vessels. Here, we have shown that the transcription factor GATA4 controls murine LSEC specification and function. LSEC-restricted deletion of Gata4 caused transformation of discontinuous liver sinusoids into continuous capillaries.

Capillarization was characterized by ectopic basement membrane deposition, formation of a continuous endothelial cell layer, and increased expression of VE-cadherin. Correspondingly, ectopic expression of GATA4 in cultured continuous ECs mediated the downregulation of continuous EC-associated transcripts and upregulation of LSEC-associated genes. The switch from discontinuous LSECs to continuous ECs during embryogenesis caused liver hypoplasia, fibrosis, and impaired colonization by hematopoietic progenitor cells, resulting in anemia and embryonic lethality. Thus, GATA4 acts as master regulator of hepatic microvascular specification and acquisition of organ-specific vascular competence that are indispensable for liver development. The data also establish an essential role of the hepatic microvasculature for embryonic hematopoiesis.

References

1. C. Géraud et al., “GATA4-dependent organ-specific endothelial differentiation controls liver development and embryonic hematopoiesis,” J. Clin. Invest., Feb. 2017

Transcriptomic analysis of acute and chronic liver injury reveals an inflammation / ER stress dependent suppression of metabolic gene networks

P. Godoy[1], G. Campos[1], W. Schmidt Heck[2], J. de Smedt[3], D. González[1], P. Nell[1], D. Feuerborn[1], S. Schlütter[1], A. Sachinidis[4], D. C Hay[5], J. G. Hengstler[1]

[1]IfADo-Leibniz Research Centre for Working Environment and Human Factors at the Technical University Dortmund, Dortmund, Germany
[2]Leibniz Institute for Natural Product Research and Infection Biology e.V.-Hans-Knöll Institute, Department/ Research Institute, University, Jena, Germany
[3]Stem Cell Institute, Leuven, Belgium
[4]Institute of Neurophysiology, Medical Faculty, University of Cologne, Cologne, Germany
[5]MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, United Kingdom, EH16 4UU

godoy@ifado.de

Introduction

Understanding transcriptional regulatory networks (TRNs) in liver disease is fundamental for risk assessment, diagnostic and therapeutic approaches. Experimental models of acute injury enable detailed examination of TRNs. However, it is not known whether the molecular principles in such models represent stereotypic processes of chronic human liver disease. Here, we used transcriptomics, bioinformatics and molecular analyses to unveil principles of TRNs in liver disease. Gene set enrichment and ingenuity pathway analysis were applied to fuzzy clustering-curated transcriptomics data from mouse liver after acute injury by CCl_4, lipopolysaccharide and tunicamycin exposure, experimental hepatocellular carcinoma (HCC), and human chronic liver disease (non-alcoholic fatty liver, HBV infection and HCC). TRNs and key components of the identified regulatory motifs were validated by real time PCR, western blotting and immunohistochemistry.

Results

The TRNs induced by acute CCl_4 intoxication were classified in three core motifs, namely ER stress, inflammation and suppression of mature liver functions. Gene network analysis showed that inflammation and ER stress control suppression of mature liver functions during acute injury via inhibition of transcription factors such as HNF4. These principles were also observed after lipopolysaccharide and tunicamycin intoxication, experimental HCC and human chronic liver disease. However, in chronic human liver disease inflammation was the most important mechanism controlling suppressing metabolic liver functions, and this principle was more pronounced according to disease severity. In conclusion, our analysis identified a stereotypic response whereby inflammation and ER stress cause suppression of mature liver functions in acute and chronic liver disease by altering multiple key liver TRNs.

Perspectives

The detailed identification of TRNs mediating liver damage and regeneration is fundamental to establish risk, diagnosis and therapeutic intervention. Our findings provide a detailed, time-resolved description of gene networks in mouse liver upon acute injury and regeneration, and revealed a crosstalk between inflammation and metabolic gene networks. These findings provide a blueprint for the identification of novel biomarkers of liver injury, and for interventions to improve the regenerative response. Importantly, the inflammation/metabolism crosstalk was also identified in HCC in rodents and in NAFLD, HBV infection and HCC in humans. This approach could be applicable to other organs exposed to drugs and reactive metabolites, such as the kidney, heart and lungs.

WISP1: A Potential Modulator of Inflammatory Responses in Acute Liver Injury

D. González / L. Pütter / G. Campos / K. Rochlitz / J. Hengstler / P. Godoy

Leibniz Research Centre for Working Environment and Human Factors and TU Dortmund, Germany

gonzalez@ifado.de

Liver diseases are a global burden and even though in the last years there has been a major progress in the knowledge and management of liver diseases, there is still a lot to understand. Matricellular proteins from the CCN family have emerged as multitasking intermediators and have been shown to play different roles in liver pathophysiology. These highly conserved secreted proteins specifically interact with and signal through various extracellular partners, in particular integrins, which enable them to play crucial roles in various processes including development, angiogenesis, wound healing and diseases such as fibrosis and cancer.

We have discovered WISP1 (Wnt-induced secreted protein-1) or CCN4 is induced upon intoxication and may play a protective role in liver pathophysiology. Furthermore, deletion of WISP1 leads to increased damage after acute liver injury by the hepatotoxicant CCl_4 compared with their wild type counterparts. Our in vivo experiments and histological and molecular biology analysis indicate: 1) WISP1 may mediate the repression of stress pathways such as JNK involved in the early response to damage; 2) deletion of WISP1 leads to higher expression of inflammatory cytokines and immune cell infiltration; 3) hepatocytes are not the main source of WISP1 secretion and non-parenchymal cells may play an important role in WISP1 expression-function.

In conclusion, WISP1 is an interesting novel target in acute liver damage, but further investigation is still needed

Anti-inflammatory effects of rosiglitazone in obesity-impaired wound healing depend on adipocyte differentiation

I. Goren / A. Siebert / J. Pfeilschifter / S. Frank

Pharmazentrum Frankfurt/ZAFES, Klinikum der Johann Wolfgang Goethe-Universität,
Frankfurt am Main, Germany

goren@chemie.uni-frankfurt.de

Introduction

Combined diabetes-obesity syndromes severely impair the regeneration of acute skin wounds in mouse models. Accordingly, in addition to the increased expression of transcriptional effectors of adipocyte differentiation such as Krüppel-like factor (KLF)-5 and peroxisome proliferator-activated receptor (PPAR)-γ as well as the associated expression of fatty acid-binding protein (FABP)-4, subcutaneous fat of genetically obese (*ob/ob*) mice is accompanied by an enhanced expression of pro inflammatory mediators such as the CXCL2 chemokine. Hence, the contribution of subcutaneous adipose tissue to exacerbated wound inflammatory conditions was evaluated herein.

Results

These observation in obese mice is in keeping with differentially elevated levels of KLF-5, PPAR-γ, leptin, FABP-4 and CXCL2 in *in vitro*-differentiated 3T3-L1 adipocytes. Notably, CXCL2 chemokine is restrictively expressed upon cytokine (IL-1β/TNF-α) stimulation only in mature, but not immature 3T3-L1 adipocytes. Importantly, the critical regulator of adipocyte maturation, PPAR-γ and in particularly the PPAR-γ2 isoform, was merely expressed in the final phase of *in-vitro* induced adipocyte differentiation from 3T3-L1 pre-adipocytes.

Consistently, the PPAR-γ agonist rosiglitazone suppressed the cytokine-induced CXCL2 release from mature adipocytes, but not from early 3T3-L1 adipocyte stages. The inhibitory effect of PPAR-γ activation on CXCL2 release appeared to be a general anti-inflammatory effect in mature adipocytes, as cytokine-induced cyclooxygenase (Cox)-2 was simultaneously repressed by rosiglitazone.

In accordance with these findings, oral administration of rosiglitazone to wounded obese mice significantly changed subcutaneous adipocyte morphology, reduced wound CXCL2 and Cox-2 expression and improved tissue regeneration.

Perspectives

The massive subcutaneous adipose tissue mass of obese diabetic mice potentially contributes to the burden of skin ulceration through increasing the amount of pro-inflammatory mediators' at the wound site, thereby worsen and add to the prolonged wound inflammation observed in diabetes-obesity conditions. Hereafter, the data presented here suggests that blunting the Pro-inflammatory propensity of the subcutaneous fat tissue may ameliorate healing process.

Particularly, the transcription factor PPAR-γ2 specifically expressed in mature adipocytes might provide an effective therapeutically target to reduce the production of inflammatory mediators from adipocytes and improve disturbed tissue regeneration in conditions of obesity.

MiR-29b-2 reduces Lysyl oxidase activity in primary hypertrophic scar cells

D. Harmanci[1] / A. Kocak[1] / C. Demirdover[2] / G. Guner Akdogan[3]

[1]Dept. of Molecular Medicine, Graduate School of Health Sciences, Dokuz Eylul University, Turkey
[2]Dept. of Plastic Reconstructive and Aesthetic Surgery, School of Medicine, Dokuz Eylul University, Turkey
[3]Dept. of Medical Biochemistry/ School of Medicine, Izmir University of Economics, Turkey

duyguharmanci@gmail.com

Introduction

Hypertrophic scars are characterized by erythematous and raised fibrous lesions predominantly composed of excessive amounts of collagen deposits. They typically remain within the boundaries of the initial injury. Despite many studies that have examined the pathophysiology of hypertrophic scars, the underlying causes and the best treatment modalities are still unknown. The miR29 family in humans includes hsa-miR-29a, hsa-miR-29b-1, hsa-miR-29-2, and hsa-miR-29c. Firstly, miR29a was found in Hela cells by Lagos-Quintana, followed by the subsequent discovery of miR-29b and miR29c. The particular interest of miR29 family members is due to their ability to inhibit the synthesis of extracellular matrix (ECM) proteins, especially collagens. Lysyl oxidase (LOX) family is a class of ECM crosslinking enzymes. LOX family enzymes play roles in fibrotic diseases. For this reason, the aim of this study was to determine the possible alterations on activity of LOX after miR-29b-2 inhibition. Firstly, we collected the tissue specimens and established primary normal and hypertrophic scar fibroblast cell cultures. Evaluation of miR-29 family members' gene expression was determined by using qPCR. According to qPCR results, miR-29b-2 was silenced with miR-29b-2 inhibitor in both primary cell cultures and then extracellular LOX activity was measured using LOX activity kit.

Results

Our data showed that the significant decrease in miR-29b-2 gene expression in skin hypertrophic scar tissue in comparison to healthy control skin. After miR-29b-2 transfection, extracellular LOX activity decreased in transfected hypertrophic scar fibroblast cells compared to non-transfected scar cells, whereas there was no significant alteration in LOX activity between transfected healthy fibroblast cells and non-transfected healthy fibroblast cells.

Perspectives

These data suggest that the downregulation of miR-29b-2 may play a role in the progression of hypertrophic scar formation. Besides, the crosslinking enzyme LOX activity decreased with miR-29b-2 transfection in hypertrophic scars. According to these results, LOX may be a possible target for miR-29 family and miR-29b-2 may affect processing ECM reducing LOX activity.

Keywords

Hypertrophic scar; fibrosis; miR-29 family; lysyl oxidase activity

Intact cell-cell interactions in fresh ***ex vivo*** lung tissue enables the investigation of pro-fibrotic mediators

C. Hesse[1,5] / S. Mang[2] / H.-G. Hoymann[1,5] / M. Niehof[1,5] / P. Braubach[3,5] / D. Jonigk[3,5] / M. Kühnel[3,5] / G. Warnecke[3,5] / O. Pfennig[4] / H.-G. Fieguth[4] / A. Braun[1,5] / K. Sewald[1,5]

[1]Fraunhofer Institute for Toxicology and Experimental Medicine, Hanover, Germany
[2]Boehringer Ingelheim Pharma GmbH & Co. KG, Immunology & Respiratory Diseases Research, Biberach an der Riss, Germany
[3]Hannover Medical School, Hanover, Germany
[4]KRH Clinics Hannover, Germany
[5]Member of the German Center for Lung Research (DZL), Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH) research network, Member of the Cluster of Excellence Regenerative Biology to Reconstructive Therapy (REBIRTH), Hannover, Germany

christina.hesse@item.fraunhofer.de

Introduction

Pulmonary fibrosis covers a scope of rapidly progressing lung disease, characterized by uncontrolled deposition of extracellular matrix (ECM), excessive proliferation of fibroblasts and destruction of cellular architecture of the lung, causing irreversible dysfunction of the organ. To date, the development of new therapies is hampered by the lack of animal models that do not entirely reflect all features of the disease as found in the patients. The complex 3-D microanatomical organization of precision-cut lung slices (PCLS) gives the possibility to reflect very closely the biological responses of the lung. Importantly, established cell-cell connections remain conserved over time, enabling diverse cellular interactions and immune signaling within the lung tissue slices. With the use of PCLS, the aim was to identify specific cell signaling pathways and pro-fibrotic mediators involved during pulmonary fibrosis.

Results

PCLS prepared from lungs of bleomycin-treated rats revealed up-regulation of important pro-fibrotic genes as compared to the NaCl-treated controls. Amongst others, extracellular matrix (ECM) genes, for example, fibronectin 1 was found to be 2-fold and collagen1a1 even 5-fold elevated as compared to control animals. Furthermore ECM remodeling enzymes, e.g. matrix metalloproteinase 7 (MMP7) was elevated up to 11-fold in bleomycin treated rat lungs as compared to the controls. Importantly, PCLS prepared from these animals retain this pattern in culture for 2-5 days. A comparable pattern of upregulated pro-fibrotic genes was found in human non-fibrotic PCLS stimulated with TGF-β and TNF-α. Here, MMP13 mRNA levels were elevated 2.5 to 5-fold and endothelin 1 levels were at least 2-fold increased. Furthermore, important pro-fibrotic mediators involved in fibrolysis and degradation of the extracellular matrix e.g. PAI-1 and PLAU were found to be significantly elevated in TGF-β and TNF-α stimulated PCLS as compared to the controls, while other genes, e.g. caveolin 1, were downregulated. In addition to the mRNA pattern, important pro-fibrotic mediators e.g. VEGF were found to be significantly elevated. The pro-inflammatory cytokine IL-β, involved in the initial wound repair mechanism and early fibrotic response, was significantly elevated in both experimental *ex vivo* systems.

Perspectives

Overall, PCLS provide a physiologically relevant model that circumvents drawbacks of simple single-cell cultures offering an ideal experimental system for the investigation of complex cell-cell interactions and signaling pathways involved in the disease onset and progression. We describe and compare here a novel pattern of pro-fibrotic signaling pathways and biomarkers in rat and human PCLS of two different experimental setups. Gene expression as well as cytokine production resembles important pro-fibrotic features after two days of culture in both systems. This can help to further elucidate the pathogenic mechanisms of pulmonary fibrosis and to evaluate novel anti-fibrotic drug candidates and development of new medications.

Nrf2-mediated fibroblast reprogramming drives cellular senescence by targeting the matrisome

P. Hiebert[1] / M. Wietecha[1] / M. Cangkrama[1] / E. Haertel[1] / J. Lee[2] / J. Brinkmann[3] / D. Kletsas[4] / J. Dengjel[5] / S. Werner[1]

[1]Institute of Molecular Health Sciences, ETH Zürich, Switzerland
[2]Natural Products Research Institute, Seoul National University, South Korea
[3]Institute of Virology and Cell Biology, University of Lübeck, Germany
[4]Institute of Biosciences and Applications, National Centre for Scientific Research, Greece
[5]Department of Biology, University of Fribourg, Switzerland

paul.hiebert@biol.ethz.ch

Introduction

Among the parallels between wound healing and cancer lies the profound influence of the underlying stroma. In particular, fibroblasts that have undergone senescence can influence neighbouring epithelial cells through an altered gene expression and secretion program featuring numerous mitogenic growth factors, cytokines and extracellular matrix (ECM), commonly referred to as the senescence associated secretory phenotype (SASP).

The transcription factor Nuclear Factor Erythroid 2 Like 2 (Nrf2) is regarded as the master regulator of the antioxidant response due to its ability to induce transcription of a set of genes capable of detoxifying harmful reactive oxygen species. In skin, activation of Nrf2 in keratinocytes offers protection from oxidative damage, but can also promote keratinocyte survival during cancer development. In skin fibroblasts however, little is known regarding the consequences of Nrf2 activation, representing a significant gap in our knowledge given the recognized importance of mesenchymal cells in mediating tissue repair and cancer.

Results

Transgenic mice expressing a constitutively active Nrf2 mutant (caNrf2) in fibroblasts demonstrate that activation of Nrf2 causes early onset of cellular senescence, including altered gene expression indicative of a SASP. *In vivo*, fibroblasts expressing caNrf2 promote keratinocyte proliferation leading to faster wound closure but also increased skin tumor expansion.

Factors leading to senescence are shown to be mediated through changes in signals originating from an altered ECM, including the known senescence inducer PAI-1. Furthermore, Nrf2 acts as a direct transcriptional activator of ECM components, including PAI-1. These results demonstrate that Nrf2 functions to reprogram stromal fibroblasts into senescent cells by targeting genes involved in the production and remodelling of ECM.

Perspectives

Activation of the Nrf2 pathway is considered as a promising strategy to reduce cellular oxidative stress in the hopes of preventing disease onset. These results highlight the complexity of such strategies and demonstrate the need for further consideration into the consequences of Nrf2 activation in stromal cells during skin homeostasis and disease.

Regenerative potential of adipocytes – Potential treatment for hypertrophic scars?

K. Hörst[1] / L. van den Broek[2] / S. Gibbs[2] / U. v. Fritschen[3] / S. Hedtrich[1]

[1]Institute of Pharmacy, Dept. of Pharmacology & Toxicology, Freie Universität Berlin, Berlin, Germany
[2]Department of Dermatology, VU University Medical Center, Amsterdam, the Netherlands
[3]Division Plastic Surgery and Hand Surgery, Helios Clinic Emil von Behring Berlin, Germany

katharina.hoerst@fu-berlin.de

Introduction

Hypertrophic scars can result from surgery (e.g. mastectomy) or burn wounds depending on the wound depth. Patients are not only affected by cosmetic problems because of the elevated and red appearance of the scar tissue, but also suffer from pain, pruritus and contractures. Following autologous fat grafting in plastic surgery, significant improvements in morphology and function of hypertrophic scar tissue have been observed repeatedly[1]. This phenomenon indicates crosstalk between the injected adipose tissue and the connective tissue[2]. However, the underlying mechanisms are largely unknown. Therefore, this project aims to unravel the interactions between adipose tissue and connective tissue during wound healing and to identify the cell types involved.

After isolation of adipose-derived stem cells (ASC) from adipose tissue, cells were differentiated into adipocytes over 14 days. Concurrently, primary human fibroblasts were stimulated with transforming growth factor ß1 (TGF-β1) for 72h to generate myofibroblasts. Additionally, fibroblasts were isolated from hypertrophic scar tissue. (Myo)fibroblasts were then incubated with conditioned media from ASCs or adipocytes. Following 24 h, gene and protein expression analyses of alpha smooth muscle actin (α-sma) and extracellular matrix (ECM) proteins were performed. In another approach, myofibroblasts were treated for 1h with the PPARɣ antagonist GW9662 (1 µm) followed by 24h incubation with adipocyte-conditioned medium. Since nuclear receptor PPARɣ interferes with TGF-β-signalling, the contribution of PPARɣ was tested.

Results

After 24 h, exposure to conditioned medium from adipocytes but not ASCs induced a significant downregulation of the myofibroblast marker α-sma on gene and protein level. Notably, this effect was even more pronounced in fibroblasts derived from hypertrophic scar tissue. A similar effect was observed for collagen I and III expression. When myofibroblasts were pre-treated with GW9662, no downregulation in α-sma could be induced indicating an involvement of PPARɣ and PPARɣ ligands in the conditioned medium from adipocytes.

Perspectives

Downregulation of α-sma and ECM proteins may indicate a modification of the myofibroblast differentiation state. A re- or dedifferentiation of myofibroblasts would be a possible explanation for the regeneration of hypertrophic scars. This aspect is currently under investigation.

References

1. Klinger et al.: J. Cranoiofac. Surg. 2013, 24: 1610-1615.
2. Guisantes et al.: Ann Plast Surg. 2012, 69 (5): 550-554.

Tissue-specific roles of FGF signaling during zebrafish caudal fin regeneration

C. Jahn / D. Wehner / G. Weidinger

Institute of Biochemistry and Molecular Biology, Ulm University, Ulm, Germany

christopher.jahn@uni-ulm.de

Introduction

Nature has evolved various strategies to deal with injury-inflicted tissue damage. Regeneration – the regrowth of tissue to full functionality – is one of them. In contrast to mammals, teleost fish have the ability to regenerate their appendages. Upon partial amputation of the caudal zebrafish fin a blastema is formed, which contains the proliferative progenitor cells of the regenerating tissues. During regenerative outgrowth the blastema and the overlying epidermis become compartmentalized into several zones defined by molecular markers, distinct proliferation characteristics and cell fates.

Little is known about the molecular mechanisms specifying these regions and the signaling pathways mediating their interaction. Loss-of-function experiments have shown that fibroblast growth factor (FGF) signaling is crucial for blastema formation and outgrowth[1,2]. However, the precise functions of the pathway during this regenerative process remain poorly understood. This is in particular due to the absence of genetic tools for temporally and spatially controlled manipulation of pathway activity. Here we have made use of the TetON system to tissue-specifically manipulate FGF signaling to dissect its tissue-specific functions during caudal fin regeneration.

Results

lef1(BAC)-promoter driven expression of a dominant-negative fibroblast growth factor receptor 1 (dnFgfr1) construct during regeneration resulted in reduced fin regenerate length and a dysmorphic fin growth phenotype. Immunostaining showed that the lef1(BAC)-promoter driven dnfgfr1 was expressed in the basal epidermal layer, the distal blastema, committed osteoblasts and in another subpopulation of osteoblast progenitors, presumably runx2+ (pre-)osteoblasts. Surprisingly, no effect on fin regeneration was observed when dnfgfr1 was expressed in the proximal basal epidermal layer (keratin18-promoter), in the distal blastema (7xTCF-promoter) or in committed osteoblasts (sp7-promoter) exclusively. Interestingly, expression of the dnfgfr1 in proliferating fibroblasts (her4-promoter) showed only reduced fin regenerate length without dysmorphic fin growth. I therefore propose that FGF signaling might have different roles in distinct cellular compartments of the fin regenerate. FGF signaling seem to be responsible for patterning and/or cell proliferation in a subpopulation of osteoblast progenitors, while in fibroblast-like cells it is responsible for cell proliferation.

Perspectives

Currently we are investigating the different functions of FGF signaling in her4+ fibroblasts compared to runx2+ (pre)osteoblasts in detail. The knowledge obtained by these experiments will bring us a step forward in understanding complex tissue interactions required for successful regeneration.

References

1. Poss et al., 2000, Dev Biol, 222(2), 347-358.
2. Whitehead et al., 2005, Science, 310(5756), 1957-1960.

Loss of AATF in renal epithelial cells results in accumulation of DNA damage, defective primary cilia and a degenerative kidney disease resembling human Nephronophthisis

M. Jain[1,2] / R. Kaiser[1,2] / M. Hoehne[1,2,3] / H. Göbel[4] / R. Müller[1,2,3] / T. Benzing[1,2,3] / B. Schermer[1,2,3] / K. Höpker[1,2,*] / G.Slaats[1,2,*]

[1] Department II of Internal Medicine and Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany
[2] Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany
[3] Systems Biology of Ageing Cologne (Sybacol), University of Cologne, Cologne, Germany
[4] Department of Pathology, University of Cologne, Cologne, Germany

manaswita.jain@uk-koeln.de

Introduction

Nephronophthisis (NPH) is a hereditary pediatric cystic kidney disease representing the most common genetic cause of end-stage renal disease in children and adolescents. NPH predominantly affects the kidneys, which are histo-pathologically characterized by interstitial fibrosis, tubular atrophy, tubular basement membrane disintegration, and to a varying degree by cysts that are typically localized at the cortico-medullary border. Almost all disease-causing mutations affect genes that encode for proteins localized to primary cilia. Recent human genetic studies on NPH have provided an intriguing link between ciliary signaling defects and altered DNA damage responses (DDR). In order to genetically address the important question how altered DDR signaling might contribute to NPH, we generated mice lacking Apoptosis Antagonizing Transcription Factor (AATF) in renal epithelial cells of the distal nephron.

Results

Here we show that renal deletion of *Aatf*, a critical mediator of the p53 response, causes both ciliary defects as well as an accumulation of DNA damage. Mice with a targeted deletion of *Aatf* in renal tubular cells were born normally, but developed progressive renal failure and a disease resembling human NPH. We observed development cystic and fibrotic kidneys starting from 8 weeks of age. Depletion of AATF in renal epithelial cells decreased the number of ciliated cells in 3D spheroid cultures and led to activated DNA damage signaling and accumulation of DNA double strand breaks. Mechanistically, AATF suppresses RNA:DNA hybrid formation, which typically causes DNA double strand breaks when conflicting with replication forks. In addition, we demonstrate recruitment of AATF to sites of damage, and we identified a role of AATF in DNA double strand break repair by regulating the recruitment of RAD50 and BRCA1.

Perspectives

This mouse model precisely recapitulates clinical and histological characteristics of human NPH, which suggest an etiologic role of altered DDR in NPH. We provide AATF as a regulator of primary cilia and modulator of the DDR connecting two patho-genetic concepts of NPH and NPH-related ciliopathies.

Preventing endothelial-to-mesenchymal transition by enhancing miR126-3p expression

N. Jordan[1] / E. Singh[2] / R. E. Redgrave[2] / B. Innes[1] / D. Hall[2] / H. M. Arthur[2] / C. Ward[1] / S. Ali[1]

[1]Institute of Cellular Medicine, Newcastle University, UK
[2]Institute of Genetic Medicine, Newcastle University, UK

nina.jordan@newcastle.ac.uk

Fibrosis is one of the leading hallmarks of chronic allograft dysfunction and is characterized as a pathologic tissue repair due to an accumulation of extracellular matrix. Extracellular matrix secreted by myofibroblasts remodels healthy graft tissue and leads to loss of graft function post-transplantation. Studies in fibrosis have revealed different origins of myofibroblasts in cardiac grafts. Recent evidence indicates that non-proliferating myofibroblasts, as well as originating from the bone marrow, could be derived from other cell types. This specifically includes the process of endothelial-to-mesenchymal transition (EndMT). Lineage tracing of endothelial cells allowed studies of EndMT *in vivo* and reported that 27% to 35% of myofibroblasts involved in cardiac fibrosis were endothelial in origin.

Over the last decade, miRNAs have increasingly been described as key regulators in biologic processes through repression or degradation of targeted mRNA. Their profile and potential role remains mainly undescribed in EndMT, although MiRNAs have been more widely studied in the related process of epithelial-to-mesenchymal transition (EMT). Therefore, we have sought for a MiRNAs signature and potential role in EndMT in models relevant to cardiac transplantation.

EndMT *in vivo* was investigated using lineage tracing transgenic Cdh5-Cre-ERT2; Rosa26R-stop-YFP mice. Mice expressing YFP specifically in endothelial cells underwent myocardial infarction and were sacrificed five days later. Lineage tracing in the *in vivo* mouse model revealed expression of mesenchymal markers in endothelial derived cells, indicating the presence of EndMT in cardiac fibrosis generated post-myocardial infarction.

EndMT was also modelled in human umbilical vein endothelial cells (HUVEC) by treatment with TGFβ2 (10ng/mL) and IL1β (10ng/mL). Endothelial and mesenchymal markers were analysed using immunofluorescence and quantitative PCR. Significantly decreased expression of endothelial markers such as vWF and increased level of mesenchymal markers such as fibronectin were observed by qPCR in HUVEC after 48 hours of treatment with TGFβ2 and IL1β ($p<0.05$). Similarly, immunofluorescence showed increased expression of fibronectin and decreased expression of VE-cadherin in HUVEC post-treatment at 6 days.

In parallel, miRNAs profile was assessed using an array from Ncounter technologies in HUVEC. Profile in untreated cells were compared to the cells treated with TGFβ2 and IL1β at 3, 6, 16, 24 or 48 hours. On these profiles, miR-126-3p was found to be down-regulated 24 hours' post-treatment and the decrease of miRNA126-3p expression was further confirmed by qPCR ($p<0.001$). Transfection of mimics (50nM) was performed to study the role of miR-126-3p in EndMT using lipofectamine. Over-expression of miR-126-3p in HUVEC restored the expression of CD31 and repressed the expression of fibronectin induced by TGFβ2 and IL1β treatment, protecting the cells from EndMT induction. MiRNA target studies allowed identification of 2 common targets using 4 different prediction tools.

We conclude that miR-126-3p may have a role and therapeutic potential in pathologic tissue remodelling following cardiac damage, which may include allograft injury post transplantation. Ongoing studies are investigating the localisation of miR126-3p in cardiac fibrosis using *in-situ* hybridisation and the direct target of miR126-3p using a 3'UTR luciferase reporter gene assay.

How stromal fibroblasts foster radiation resistance in human prostate cancer xenografts

J. Ketteler[1] / A. Panic[1,2] / H. Reis[3] / C. Herskind[4] / P. Maier[4] / H. Rübben[2] / V. Jendrossek[1] / D. Klein[1]

[1]Institute of Cell Biology (Cancer Research), University of Duisburg-Essen, University Hospital, Essen, Germany
[2]Department of Urology and Urooncology, University of Duisburg-Essen, University Hospital, Essen, Germany
[3]Institut of Pathology, University of Duisburg-Essen, University Hospital, Essen, Germany
[4]Department of Radiation Oncology, Univ. Hospital Mannheim, Heidelberg University, Mannheim, Germany

julia.ketteler@uk-essen.de

Introduction

The clinical relevance of the tumor microenvironment in modulating the response of solid tumors to chemotherapy and radiotherapy has been documented. Herein, the membrane protein caveolin-1 (Cav1) came into focus as it is overexpressed or mutated in solid human tumors, e.g. prostate cancer. Cav1 might regulate several signaling processes and cellular functions with significance for the survival of cancer cells including resistance-promoting interactions with extracellular matrix proteins, tumor angiogenesis and metastasis. Although there are good treatment options for benign prostate carcinoma, advanced stages still display high therapy resistance to radio- and chemotherapy.

During prostate cancer progression a characteristic switch in Cav1 expression occurs, when prostate epithelial cells gain Cav1 expression, whereas a decrease of stromal Cav1 can be observed in fibroblasts of the tumor microenvironment at advanced stages. Here, we investigated the influence of differential Cav1 expression levels on the radiosensitivity of prostate carcinoma cells as well as the influence of stromal Cav1 expression on the radiation response of prostate carcinoma cells *in vitro* and *in vivo*.

Results

Cav1-silenced PC3 cells [PC3 Cav1(-)] showed an increased sensitivity to ionizing radiation (IR) *in vitro*, while Cav1(-) fibroblasts showed increased radioresistance. In order to study the influence of stromal Cav1 for the radiation response of prostate carcinoma cells we started with an indirect approach using cell culture supernatants (SN) derived from the differentially Cav1-expressing HS5 fibroblasts. Here, epithelial PC3 cells cultured with HS5 Cav1(-) SN resulted in an increase in radioresistance. This results could be confirmed *in vivo* by implanting PC3 Cav1(-) cells with differential Cav1-expressing fibroblasts.
Tumors derived from PC3 (-)HS5(-) cells showed significantly increased growth after IR and thus an increase in radioresistance as compared to tumors derived from PC3(-)HS5(+) cells. Importantly, the decreased radiation-induced growth delay in tumors derived from PC3(-)HS5(-) cells was associated with an increased reactive tumor stroma. Indication of radioresistance in advanced tumor stages could also be demonstrated by analyzing human prostate tissue for Cav1 and reactive stroma markers.

Perspectives

Conclusively, the radiation response of human prostate tumors is critically regulated by Cav1 expression in stromal fibroblasts. Loss of stromal Cav1 expression in advanced tumor stages may thus contribute to resistance of these tumors to radiotherapy. In the current work we aim to specify the mechanisms how Cav1-deficient fibroblasts mediate the radioprotective effects. We investigate the pathways that are alternately regulated in prostate carcinoma cells by differential Cav1-expressing fibroblasts and try to identify decisive signaling molecules in the secretome of differential Cav1-expressing stromal cells.

Generic wound signals initiate regeneration in a missing-tissue context

F. Klenner[1] / S. Owlarn[2] / K. Bartscherer[2] / G. Weidinger[1]

[1]Institute of Biochemistry and Molecular Biology, Ulm University, Germany
[2]Max Planck Research Group Stem Cells & Regeneration, Max Planck Institute for Molecular Biomedicine, Münster, Germany

felix.klenner@uni-ulm.de

Introduction

All higher organisms have evolved mechanisms to repair damage after wounding, but the capacity to regenerate complex organs varies widely between species. One fundamental question in regeneration research is whether regeneration is triggered by the same molecular signals that trigger wound healing, or whether the initial wounding response already differs between regenerating and non-regenerating organs. We have addressed this question using the caudal fin regeneration model of the zebrafish (*Danio rerio*). Specifically we asked whether skin wounds that normally do not trigger regeneration can do so in a context where tissue is already "missing".

Result

To create such a missing-tissue context, we amputated fins and allowed the epidermis to heal, but blocked regeneration. This was achieved by inhibition of FGF signaling using overexpression of a dominant-negative FGF receptor 1 in heatshock inducible transgenic fish (Lee et al., 2005. *Development* 132, 5173-5183). By applying four heat shocks daily until 8 days post amputation (dpa), we induced a stable block of regeneration that was still present 12 days after the heatshock treatment had been stopped (20 dpa). To test whether fin rays had retained their regenerative potential under such blocking conditions, we re-amputated individual rays following a recovery period of 5 days after the end of the heatshock treatment. Re-amputated rays robustly regenerated, showing that blocked fins were in a "dormant" state and still able to regenerate. This model allowed us to test which types of wounds can initiate regeneration in a missing tissue context. We developed a protocol to ablate the distal epidermal tip of an individual blocked ray without disturbing neighboring rays or damaging bone tissue.

Such epidermal injuries did not initiate regeneration in non-amputated fins. In contrast, epidermal wounding was followed by blastema formation and regenerative outgrowth in dormant rays. We further tested dormant fins, in which some rays had been amputated in a more proximal position. Again, FGF signaling inhibition stably blocked ray regeneration, but allowed skin healing, thereby generating "skin fields" devoid of bony structures between the stump of the amputated ray and the amputation plane of neighboring rays. Small incisions into such skin fields could induce a regenerative response of bony tissues in the dormant stump of these rays. Strikingly, we found that also in planarians incision wounds that normally do not induce regeneration can do so in a missing-tissue context. We therefore conclude that regeneration can be triggered by generic wound-induced signals if they occur in a missing-tissue context. Thus, the ability to regenerate might not depend on specialized wounding-induced signals, but rather on the interaction of generic wounding signals with molecular systems that detect the absence of tissue.

Perspectives

The stable missing tissue context is a useful tool to investigate the nature of these signals. Gene expression profiling approaches in early phases after wounding will be performed to investigate which signals are responsible for healing or regeneration and how the organism decides between the two processes. This is of special importance when asking about why some organisms do have the ability to regenerate and others do not.

Epigallocatechin-3-gallate protects against oxidative stress in scleroderma animal model

A. Kocak[1] / D. Harmanci[1] / M. Birlik[2] / G. Guner Akdogan[3]

[1]Dept. of Molecular Medicine / Graduate School of Health Sciences, Dokuz Eylul Univ., Izmir, Turkey
[2]Dept. of Rheumatology and Immunology/ School of Medicine, Dokuz Eylul University, Turkey
[3]Dept. of Medical Biochemistry/ School of Medicine, Izmir University of Economics, Turkey

kocak.ayse@gmail.com

Introduction

Systemic sclerosis or scleroderma (SSc) is an autoimmune multisystemic connective tissue disease characterized by skin and internal organ fibrosis. Underlying mechanism is still unclear in scleroderma. Although there is no specific treatment for claleroderma, various treatments may alleviate symptoms and improve the quality of life. Epigallocatechin-3-gallate (EGCG) is a phenol with antioxidant effects in many disease processes.

The aim of this study was to investigate the antioxidant effects of epigallocatechin-3-gallate in the scleroderma process in experimental mouse model with bleomycin. For this purpose, thirty-two healthy female Balb-c mouse species (22 ± 5 g) were used in the experiment and were randomly divided into four groups: control (n = 8), bleomycin (n = 8), bleomycin + EGCG EGCG (n = 8).

At the end of the experiment, bleomycin applied skin tissues were collected. Sodium dismutase enzyme (SOD) and malondialdehyde (MDA) levels have been analyzed for oxidative stress. High performance liquid chromatography (HPLC) was used for MDA measurements. Colorimetric kit was used for SOD analysis. Furthermore, the ratio of phosphorylated p-38 / total p-38 protein, which is an oxidative stress parameter, and phosphorylated p-Akt / total p-Akt protein, which plays a role in cellular survival in signal pathways, was measured by western blotting.

Immunohistochemistry (α-smooth muscle actin) and histochemistry (masson trichrome and hematoxylin & eosin) studies were also performed on formalin-fixed paraffin-embedded skin samples to confirm the validation of the experimental model and possible inter-group changes.

According to Western blotting results, PP-38 decreased in EGCG group and PP-Akt increased in EGCG group. It has been shown that EGCG can support treatment in scleroderma.

Results

When the experimental and control groups were compared, the degree of fibrosis in the connective tissue of the dermis areas stained with masson trichrome decreased in the experimental groups (group treated with EGCG). Again, in the EGCG groups, there was a significant reduction in fibrosis in the area of the dermal surface according to hematoxylin & eosin measurements.

SOD activity was increased in the EGCG groups compared to the positive control group, and MDA was significantly decreased in the EGCG groups.

According to Western blotting results, pp-38 decreased in EGCG group and pp-Akt increased in EGCG group. It has been shown that EGCG can support treatment in scleroderma.

Perspectives

These data suggest that EGCG may play a role in the skin fibrosis via p-38 and pp-Akt signaling pathway.

Keywords

Scleroderma, fibrosis, oxidative stress

Ablation of epidermal collagen chaperoning results in dermal fibrosis

A. Köhler[1] / M. Mörgelin[2] / W. Bloch[3] / M. Aumailley[1] / F. Zauke[4] / U. Baumann[5] / G. Sengle[1,6]

[1]Center for Biochemistry, Medical Faculty, University of Cologne
[2]Department of Clinical Sciences, Division of Infection Medicine, Lund University
[3]German Sport University Cologne
[4]Dr Rolf M Schwiete Research Unit for Osteoarthritis, Orthopedic University Hospital, Friedrichsheim gGmbh, Frankfurt/Main
[5]Institute of Biochemistry, Faculty of Mathematics and Natural Sciences, University of Cologne
[6]Center for Molecular Medicine Cologne, University of Cologne

a.koehler@uni-koeln.de

Introduction

Heat-shock protein 47 (hsp47) is an essential chaperone during procollagen biosynthesis which stabilizes the trimeric state of procollagen molecules and is thereby required for proper collagen secretion[1]. Collagen type I and several other fibrillar collagens were shown to be major clients of hsp47 indicating its essential role for collagen-rich connective tissues such as bone, cartilage and skin. Mutational inactivation in humans or genetic ablation in mice showed that hsp47 is indispensable for bone[2] and cartilage[3] formation during development, but also specific ablation in the dermis leads to embryonic lethality[3]. Since we found that hsp47 interacts with transmembrane collagens of the epidermis we wanted to investigate the functional consequence of keratinocyte-specific genetic ablation of hsp47 in mice.

Results

We observed a severe fibrotic phenotype in adult male Hsp47 $^{fl/fl}$; K14Cre animals. Hsp47 $^{fl/fl}$; K14Cre animals showed dermal and epidermal thickening, alterations in collagen fibril ultrastructure as well as in the organization of collagen fibrils within the dermis.

Perspectives

Our surprising findings implicate an essential role for hsp47 in skin homeostasis which when perturbed results in impaired epidermal-dermal crosstalk.

References

1. Ishida, Y. *et al.* Type I Collagen in Hsp47-null Cells Is Aggregated in Endoplasmic Reticulum and Deficient in N-Propeptide Processing and Fibrillogenesis. *Mol Biol Cell* 17, 2346–2355 (2006).
2. Christiansen, H. E. *et al.* Homozygosity for a missense mutation in SERPINH1, which encodes the collagen chaperone protein HSP47, results in severe recessive osteogenesis imperfecta. *Am. J. Hum. Genet.* 86, 389–398 (2010).
3. Masago, Y. *et al.* The molecular chaperone Hsp47 is essential for cartilage and endochondral bone formation. *J. Cell. Sci.* 125, 1118–1128 (2012).

Identification of NASH-associated signaling networks in murine hepatic stellate cells

A. Marcher[1] / S.M. Bendixen[1] / M.K. Terkelsen[1] / C. Wiwie[2] / J. Baumbach[2] / R. Röttger[2] / K. Ravnskjær[1]

[1]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Denmark
[2]Department of Mathematics and Computer Science, University of Southern Denmark, Denmark

ravnskjaer@bmb.sdu.dk

The hepatic fibrosis score is the strongest predictor of disease-specific mortality in patients with non-alcoholic fatty liver disease. Hepatic fibrosis occurs as the disease progresses to non-alcoholic steatohepatitis (NASH) with hepatic inflammation and the appearance of fibrogenic myofibroblasts.

Activated hepatic stellate cells (HSCs) are the major source of collagen-producing myofibroblasts in NASH. Understanding the molecular pathways by which quiescent HSCs are activated and reprogrammed into myofibroblasts during NASH progression is hence key to improved diagnosis and targeted intervention in the development of fibrosis.

Here we present networks of NASH-associated factors in primary HSCs isolated from Western diet-fed mice. These factors and associated networks were identified using RNA-sequencing, novel time-course clustering algorithms, and network enrichment analyses. By topographically comparing changes in gene expression profiles across three experimental models of HSC activation we found core signaling nodes linking external signals to the reshaping of HSC morphology and function.

These regulated core nodes include cell surface receptors, putative signal transduction complexes, as well as downstream effectors. The significance of these as necessary drivers of HSC reprogramming is currently being validated experimentally.

This study demonstrates the power of combining time- and cell type-resolved transcriptomic analyses across different experimental models of HSC activation. It furthermore shows the usefulness of time-course clustering algorithms for identifying *conserved*, disease-associated gene sets with near-identical expression trajectories.

Our findings expose regulatory networks associated with phenotypic changes in primary murine HSCs during NASH development. Novel putative drivers of HSC reprogramming to hepatic myofibroblasts are currently being validated experimentally and could become attractive candidates for pharmacological intervention in the development of hepatic fibrosis.

Collagens modulate sensitization signaling and CGRP expression of nociceptive neurons

K. Moeller[1] / C. Loos[2] / J. Isensee[1] / J. Hasenauer[2] / T. Hucho[1]

[1]Experimental Anaesthesiology and Pain Research, Department of Anaesthesiology and Intensive Care, University Hospital of Cologne, Cologne, Germany
[2]Institute of Computational Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

katharina.moeller@uk-koeln.de

Many painful tissue alterations such as wounding or tumor development are accompanied by modifications of the extracellular matrix (ECM). The direct impact of ECM proteins on sensory neurons has been studied extensively in respect to regeneration and neurite outgrowth. However, knowledge about their influence on pain sensitization signaling remains sparse. Thus, we aimed to identify ECM proteins which alter sensitization signaling in primary nociceptive neurons, and subsequently to analyze their neuronal subgroup specificity and cellular mechanism.

Dissociated dorsal root ganglia were cultured overnight in 96-well plates coated with various ECM proteins and stimulated for several durations with classical pain sensitizing substances. Via immunocytochemistry phosphorylation levels of endogenous PKA-subunits and Erk1/2 were labelled and quantified via the application of High-Content Screening (HCS) microscopy and software based single-cell analysis. Single-cell data of activation kinetics and dose responses was then used to construct a mechanistic computational model, which was analyzed for mechanistic changes by “ordinary differential equation constrained mixture modelling (ODE-MM)”. Subsequently, ECM hits were examined for subgroup comparability and specificity, and the cellular mechanism using pharmacological tools and immunocytochemistry.

Our screen analyzed circa one million neurons for potential changes of 17 ECM proteins on nociceptor sensitization signaling. We tested for alterations in basal and ligand-induced (NGF-, 5-HT, and Oncostatin M (OSM)) Erk1/2 or PKA phosphorylation levels at seven time points resulting in 510 tested conditions. We identified various members of the collagen family to specifically modulate NGF-induced amplitude of Erk1/2 but not of PKA kinetics. In contrast, none of the other tested ECM proteins altered basal Erk1/2 or PKA phosphorylation or 5-HT or OSM-induced Erk/PKA kinetics. Subgroup analysis in combination with ODE-MM validated neuronal subgroup comparability. Mechanistic analysis identified NGF-mediated effects on TrkA receptor level to be the reason for differential pErk1/2 signaling. Furthermore, we could show that collagens induce long-lasting elevations of pErk1/2 levels and CGRP expression in sensory neurons.

By this first screen using a High-Content Screening microscopy approach on circa one million primary sensory neurons for factors modulating sensitization signaling we identify collagens to increase specifically NGF-induced Erk1/2 pain sensitization signaling. Since collagens are especially upregulated or altered in wounds and tumors, our results suggest this specific group of extracellular matrix proteins to augment nociceptor sensitivity in these highly painful conditions. The observed elevated CGRP expression proposes a further increased pain signaling by para- and autocrine CGRP activity.

Cellular expression of TLR2 is positively associated with cellular proliferation and apoptosis as well as VEGF expression in HCC

F. Mohamed[1,2] / S. Hammad[1,3] / ***A. Winstanley[4]*** / T. ***Luong[5]*** / S. Dooley[1#] / R. Jalan[6#]

[1]Molecular Hepatology Section, Dept. of Medicine II, Medical Faculty Mannheim, Heidelberg Univ., Mannheim, Germany
[2]Dept. of Pathology, Faculty of Medicine, Minia Univ., Minia, Egypt
[3]Dept. of Forensic Medicine and Veterinary Toxicology, Faculty of Veterinary Medicine, South Valley Univ., Qena, Egypt
[4]Dept. of Cellular Pathology, University College Hospital, London, UK
[5]Dept. of Cellular Pathology, Royal Free London NHS Foundation Trust, London, UK
[6]UCL Institute for Liver and Digestive Health, Royal Free Hospital, London, UK
# Equal contribution

fatma.mohamed@medma.uni-heidelberg.de

Introduction and Aim

Unlike toll like receptor 7 and 9, the roles of toll like receptor 2 (TLR-2) during chronic liver diseases and hepatocellular carcinogenesis are not well understood. The aim of this study was to characterise both nuclear and cytoplasmic expression of TLR-2 along different chronic liver disease settings including viral hepatitis, cirrhosis and HCC. Then, these findings were correlated with cellular proliferation and apoptosis as well as tissue vascularisation.

Material and methods and Results

We used human tissue array platforms, which included 8 normal livers, 19 hepatitis, 21 cirrhosis and 42 HCC patient liver samples. Immunohistochemistry was performed for expression of TLR-2, Ki-67, cleaved caspase 3 and VEGF, to characterize receptor expression and translocation, cell proliferation, apoptosis and vascularization. The scoring was performed in a blinded fashion by two independent pathologists. Cytoplasmic expression was scored - depending on staining intensity - into weak (+) moderate (++) and strong (+++). For quantification of Ki-67 expression, positively stained nuclei among 1000 hepatocytes were counted in the highest expression area using a standardized grid.

Cytoplasmic TLR-2 expression was weak (+) in 5/8 normal liver cases, 10/19 hepatitis cases and 8/21 cirrhosis patients. Moderate to strong expression was found in some cases of hepatitis and cirrhosis. Nuclear and cytoplasmic TLR-2 expression was recorded in HCC with weak (+) intensities in 12/42 cases and moderate to strong stainings (++ and +++) in 19/42 cases. 11 cases were negative (Table 1).

TLR2	Normal n=8	Hepatitis n=19	Cirrhosis n=21	HCC n=42
Nuclear	0 (0%)	2 (10.5%)	3 (14.3%)	13 (30.9%)
Cytoplasmic	5 (+)	10 (+) 2 (++)	8 (+) 3 (++) 3 (+++)	12 (+) 13 (++) 6 (+++)

Surprisingly, both cytoplasmic and nuclear TLR-2 expression were significantly correlated with proliferative index (r=0.24 and 0.37), apoptotic score (r=0.27 and 0.38) and vascularization (r=0.56 and 0.23) in HCC. These results show -for the first time- a positive correlation of TLR-2 with cancer hallmarks including proliferation, apoptosis and vascularization in HCC. We also found that nuclear TLR-2 translocation in HCC, in contrast, the cytoplasmic expression in hepatitis and cirrhosis.

Perspectives

Our data suggest that TLR-2 might have a potential role in HCC progression and this effect could be driven by cellular proliferation, apoptosis and vascularisation.

Endothelial cell-derived MMP-14 is dispensable for skin formation and repair

S. Neumann / M. Kümper / C. Mauch / P. Zigrino

Department of Dermatology, University of Cologne, Cologne, Germany

maike.kuemper@uk-koeln.de

Angiogenesis, the formation of new blood vessels from pre-existing ones, is a natural and crucial process during development and in adulthood. The membrane-type 1 matrix metalloproteinase (MMP-14) belongs to a large family of zinc-dependent endopeptidases shown to play a critical role in angiogenic processes. As result of MMP-14 deficiency, mice display severe skeletal defects and die within the first three weeks of birth.

To investigate the cell-autonomous role of MMP-14 expression in endothelial cells, we generated a conditional knockout mouse line with endothelial cell-specific depletion (Tie2-driven Cre) of MMP-14 (MMP-14 $^{EC-/-}$). Mice carrying the endothelial cell-specific MMP-14 deletion are mostly normal and have a normal life span. The skull of MMP-14 $^{EC-/-}$ mice at postnatal day 7 displayed slightly delayed suture closure, a lightly domed skull and a shortened snout, similar as observed in the complete MMP-14 knockout and previously accredited to the defective MMP-14 dependent dissolution of non-mineralized cartilage by osteoblast. Strikingly our data demonstrate that this phenotype can result from ablation of MMP-14 in endothelial cells, thus revealing a crucial role for endothelial MMP-14 in membranous ossification and suture closure. Adult mice (3 months) also developed shortened snouts with deviations of the midface. However, long bones (femurs) displayed no differences in ossification or in the formation of a marrow cavity.

Histological analysis of the skin revealed an overall normal skin morphology and epidermal differentiation patterns. Wound repair in MMP-14 $^{EC-/-}$ followed the same kinetics as in wild type mice and re-epithelialization, epidermal differentiation and granulation tissue formation were comparable. Immunohistological analysis further showed no altered neovascularization (CD31/SMA stainings) at day 5 and day 17, in this last analyzed time point, collagen density and fibers were comparable to control mice. To investigate whether single MMP-14 deficient endothelial cells showed molecular and functional defects ascribable to lack of MMP-14 when grown in a simplified dermis-like culture system *in vitro*, we embedded isolated endothelial cells as single cells in a three-dimensional fibrillar collagen matrix and induced formation of a vascular network with various stimuli. In this culture system, isolated MMP-14 $^{EC-/-}$ endothelial cells formed tube-like structures similar to controls, however these structures did not penetrate the gels well.

In addition, we detected large areas of clustered and flattened cells, not invading the matrix, in the absence of MMP-14. Taken together, with these studies we could show that *in vivo* depletion of MMP-14 in endothelial cells whereas important for membranous ossification and suture closure in skulls development, is dispensable and possibly compensated during skin developmental processes and homeostasis.

Fibroblast secretion of TGFβ1 is executed by secretory autophagy

J. Nüchel[1] / S. Ghatak[2] / A. Zuk[1], M. Mörgelin[3] / T. Krieg[2,4,5] / G. Sengle[1,4] / B. Eckes[2] / M. Plomann[1]

[1]Biochemistry II, University of Cologne, Cologne, Germany
[2]Dermatology, University of Cologne, Cologne, Germany
[3]Division of Infection Medicine, Lund University, Lund, Sweden
[4]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Germany
[5]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD)

nuechel.julian@uni-koeln.de

TGFβ1 is a pleiotropic cytokine with cell type-specific effects modulating growth, survival and differentiation. It has been shown to play key roles in tissue fibrosis and tumorigenesis. While signaling from different TGFβ receptors and extracellular activation of proTGFβ1 are well understood, information on intracellular trafficking of the growth factor and its secretion is sparse.

Here we deciphered the molecular mechanism of TGFβ1 secretion by fibroblasts. Our previous work demonstrated severely impaired secretion of TGFβ1 despite normal bulk secretion by fibroblasts deficient in either integrin β1 or integrin-linked kinase (ILK), which correlated with attenuated fibrotic responses in mice in vivo. ILK-null fibroblasts further exhibited abnormally high RhoA/ROCK activity, inhibition of which normalized TGFβ1 secretion.

We now show that high RhoA activity is due to dysfunction of GRAF1, a Rho-GAP, whose activity is distinctly enhanced by binding to ILK. Accordingly, silencing of GRAF1 reproduces the ILK-null phenotype with high RhoA and low Rac1 activity and severely impaired TGFβ1 secretion. GRASP55, involved in regulating Golgi architecture and in selecting cargo for trafficking, was found to co-localize with proTGFβ1 in the Golgi and in Golgi-derived vesicles, and these structures were positive for the autophagosomal marker LC3B. Of note, ultrastructural analysis revealed the accumulation of proTGFβ1 in double-walled autophagosomes. GRASP55 was able to directly interact with mammalian Atg8-like proteins (LC3s and GABARAPs) via a LC3-interacting motif. Mutation of this motif led to an intracellular accumulation of TGFβ1 and thereby reduced secretion.

These results together with a role of GRAF1 in the formation of autophagosomes suggested a link between autophagy and TGFβ1 secretion. Silencing GRAF1 or ILK in human fibroblasts indeed strongly reduced autophagy. Moreover, abrogating autophagy in fibroblasts (ablation of ATG5, ATG7 or Beclin-1) blocked TGFβ1 secretion. Interestingly, this unusual secretion mode "secretory autophagy" for proTGFβ1 is not restricted to fibroblasts but operates also in murine and human macrophages. How secretory autophagosomes are transported to the plasma membrane is not fully understood, but involves RAB8A.

Thus, besides their well-documented role in proTGFβ1 activation in the extracellular space, integrins are essential for controlling intracellular TGFβ1 transport and secretion through their link to ILK.

Generic wound signals initiate regeneration in missing-tissue contexts

S. Owlarn[1] / F. Klenner[2] / D. Schmidt[1] / F. Rabert[1] / G. Weidinger[2] / K. Bartscherer[1]

[1]Max Planck Research Group Stem Cells and Regeneration, Max Planck Institute for Molecular Biomedicine, University of Münster, Germany
[2]Institute of Biochemistry and Molecular Biology, Ulm University, Germany

suthira.owlarn@mpi-muenster.mpg.de

Introduction

Regeneration is widely distributed throughout the animal kingdom yet how this process is initiated remains enigmatic. Different responses are induced following wounds that involve tissue loss and those that do not, for instance blastema formation, which occurs exclusively at regenerating wounds. Our study aims at understanding the cellular and molecular basis of this distinction.

Results

Here, we show that generic wound-induced signals mediated by the conserved Extracellular-signal regulated kinase (ERK) pathway interact with the tissue context to determine whether an injury leads to regeneration. Inhibition of ERK activation in the highly regenerative planarian *Schmidtea mediterranea* prevented regeneration-associated changes in stem cell dynamics, blastema formation and tissue remodeling in response to amputation. Strikingly, regeneration was re-started after incisions that did not involve further tissue loss. Our results demonstrate that generic wound-induced signals are necessary for regeneration initiation and that the surrounding context determines whether these signals lead to a regenerative response. This seems to be a general principle, as regeneration was also re-started after small wounds in zebrafish fins where regeneration was previously blocked by FGFR1/MAPK/ERK inhibition.

Perspectives

Our results from planarians and zebrafish demonstrate that regeneration requires both MAPK/ERK-mediated wound signals and positional discrepancy within the target tissue. The ability to interpret wound-induced signals in a missing-tissue context might therefore be one of the crucial differences that determine whether an animal can regenerate.

Role of fibroblast MMP-14 in skin homeostasis and melanoma growth

E. Pach[1] / J. Brinckmann[2] / A. Niehoff[3] / Y. Lu[4] / N. Giebeler[1] / B. Eckes[1] / K. E. Kadler[3,4] / C. Mauch[1] / P. Zigrino[1]

[1]Department of Dermatology, University of Cologne, Germany
[2] Department of Dermatology,University of Lübeck, Germany
[3]Institute of Biomechanics and Orthopaedics, German Sport University, University of Cologne, Germany
[4] Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, University of Manchester, UK

elke.pach@uk-koeln.de

MMP-14 is a membrane bound matrix metalloprotease that coordinates breakdown of extracellular matrix during tissue remodelling. Mice lacking endogenous production die by 3 weeks of age and present skeletal abnormalities and delayed postnatal growth.

To avoid early lethality and to analyze the distinct function of fibroblast MMP-14 in adult skin homeostasis we generated mice with inducible deletion of MMP-14 in the dermal fibroblasts (MMP-14$^{Sf-/-}$). To our surprise when deletion of MMP-14 was induced, mice were smaller than control littermates. Moreover, the animals developed a fibrotic skin phenotype with increasing up to twofold thickness of the dermal connective tissue. Along with levels of collagen type I, stiffness and tensile strength increased, while collagen cross-links were unaltered. In vitro, MMP-14$^{Sf-/-}$ fibroblast did not display significant enhancement of collagen *de novo* synthesis, but collagen type I accumulated as a result of loss of collagenolysis by MMP-14$^{Sf-/-}$ fibroblasts. However, bleomycin-induced fibrosis in skin proceeded in a comparable manner in controls and MMP-14$^{Sf-/-}$, but resolution was impaired in MMP-14$^{Sf-/-}$.

As we know that tumor growth strongly depends on the interaction of tumor cells with their microenvironment including the surrounding connective tissue we were interested to learn whether alteration of the dermal connective tissue could alter proliferation of tumor cells. Surprisingly, grafting melanoma cells to the collagen rich stiff dermis of these mice resulted in decreased tumor growth. This was paralleled by reduced numbers of lymphatic and blood vessels around the invaded tumors in addition to reduced proliferation of melanoma cells, while inflammatory responses were not altered. Altogether, loss of MMP-14 activity in fibroblasts results in enhanced tissue density and tension which in turn inhibit melanoma growth by attenuating angiogenesis and tumor cell proliferation.

Taken together, these data indicate that MMP-14 expression in fibroblasts plays a crucial role in collagen remodeling in adult skin and largely contributes to dermal homeostasis underlying its pathogenic role in fibrotic skin disease and during melanoma growth.

Function of the secreted tyrosine kinase VLK in liver injury and regeneration – Extracellular phosphorylation as a potential regulatory mechanism of matrix remodeling

S. Pantasis[1] / R. Graf[2] / S.Werner[1] / M. Bordoli[1]

[1]Institute of Molecular Health Science, ETH Zurich, Switzerland
[2]Departement of Surgery and Transplantation, University Hospital Zurich, Switzerland

sophia.pantasis@biol.ethz.ch

Introduction

The first characterized secreted tyrosine kinase, vertebrate lonesome kinase (VLK), is responsible for the phosphorylation of a broad range of secretory pathway-resident and extracellular matrix (ECM) proteins. Although a wide variety of extracellular proteins have been reported to be tyrosine phosphorylated *in vivo*, the biological significance of extracellular tyrosine phosphorylation and its potential role in regulating ECM dynamics are, to date, completely unknown.

We hypothesize that VLK-dependent extracellular tyrosine phosphorylation plays a crucial role during the extensive ECM rearrangement taking place in physiological processes like wound healing or organ regeneration, as well as in diseases like fibrosis or metastasizing tumors. Here, we focus on liver regeneration following acute or chronic liver injury, a process known to be extensively driven by ECM remodeling.

Results

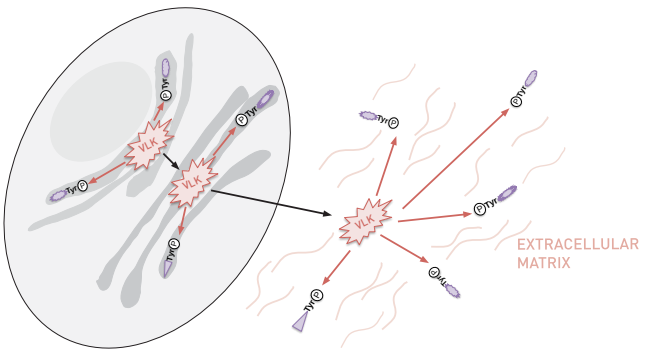
We showed VLK co-localization with cytokeratin 19 in bile ducts of the liver and pancreatic ducts. Moreover, following acute liver injury induced by CCl_4 injection, VLK expression was detected at sites of ductular reactions. Interestingly, although VLK seems to be expressed to a significant lower level in hepatocytes, its expression in cultured primary hepatocytes changed concomitantly with the increase in expression of bile duct markers, specifically *Sox9* or *Yap*.

The secreted protein osteopontin is among the most interesting identified VLK substrates. VLK-dependent tyrosine phosphorylation in osteopontin occurs at a highly-conserved integrin binding site, located next to an MMP cleavage site. Osteopontin co-localizes with VLK to bile ducts and cleaved osteopontin has been shown to favor neutrophil recruitment during inflammation in several models of liver injury. Our current efforts aim to understand how VLK-dependent phosphorylation affects osteopontin processing in bile duct cells.

Perspectives

To further investigate the biological function of VLK and extracellular tyrosine phosphorylation in the context of liver regeneration, we plan to generate a liver specific VLK knock-out in mice using the Cre-Lox system. Mice will then be subjected to acute or prolonged liver injury to monitor the physiological role of VLK in the regenerative process. Our downstream analysis will focus on the evaluation of parameters like necrosis, inflammation, cellular proliferation, as well as ECM composition and structure. Furthermore, *in vitro* studies will focus on the identification of novel VLK substrates in primary bile duct cells and on the downstream effects of VLK-dependent phosphorylation.

Summarizing, our studies aim to establish extracellular tyrosine phosphorylation as a potential regulatory mechanism of processes characterized by ECM remodeling, providing a basis for future novel therapeutic applications.



Role of astacin-like Proteinases in Wound Healing, Scarring and Fibrosis

F. Peters[1] / D. Kruppa[2] / A. Otte[1] / C. Moali[3] / V. Dive[4] / A. Nyström[2] / C. Becker-Pauly[1]

[1]Biochemical Institute, Christian-Albrechts-University of Kiel, Germany
[2]Department of Dermatology, University Medical Center, Freiburg, Germany
[3]Institut de Biologie et Chimie des Protéines, Lyon, France
[4]CEA, Section of Molecular Engineering of Proteins (SIMOPRO), Labex LERMIT, Gif/Yvette, France

fpeters@biochem.uni-kiel.de

Introduction

Physiological wound healing is a highly regulated process including the expression and activity of proteases. Dysregulation of these enzymes can result in enhanced extracellular matrix generation and fibrotic diseases as well as chronic wounds. Meprin α, meprin β and BMP-1 are members of the astacin family of metalloproteases and expressed in different compartments of the skin. Astacin proteases are well known to modulate extracellular matrix proteins, such as collagens I and III, and increased meprin expression is associated with increased collagen deposition in different fibrotic conditions. Aim of this study is to investigate the roles of astacin-like proteinases in wound healing and scarring.

Results

For epidermal effects, the HaCaT keratinocyte cell line was used for initial in vitro studies. Treatment with TGFβ1, an important cytokine for cell proliferation and differentiation, resulted in upregulation of all three astacins on mRNA level. Immunofluorescence staining of scratched cells showed meprins and BMP-1 localization at the border of migrating cells indicating a role for ECM modulation at the 'wounded' area. Interestingly, reduced 'wound closure' was observed when using recombinant active proteases as well as meprin inhibitors revealing a tight regulation of proteases' activities during epidermal regeneration. Meprin β was shown to be overexpressed in the fibrosis developing Fra2 transgenic mouse model. Long-term *in vivo* application of a specific meprin β inhibitor reduced collagen deposition in skin and confirmed the role of meprins in fibrotic progression.

Perspectives

The establishment of a 3D skin equivalent is currently in progress. Changes in skin structure will be investigated by a knockout of meprins in keratinocytes *via* CRISPR/Cas9. Additionally, cells will be treated with recombinant active protease or specific meprin inhibitors during differentiation. Furthermore, different mouse models will be used to study the role of meprins *in vivo.*

Wound healing experiments of meprin α and meprin β knockout mice are under investigation to ascertain their role in physiological wound healing. Additionally, mice overexpressing meprin α or meprin β in the Rosa26 locus will be crossed to KRT5-Cre and Col1a2-Cre mice to induce fibrosis and skin disorders. Finally, new therapeutic strategies by inhibition of astacin-like proteinases will be designed for the treatment of fibrotic conditions.

PAI-1 mediates Mast Cell-Fibroblast Interactions in a Mouse Model of Fibrosis

N. Pincha[1, 2] / E. Y. Hajam[1] / K. Badarinath[1] / S. P. R. Batta[1] / C. Jamora[1]

[1]IFOM-inStem Joint Research Laboratory, Centre for Inflammation and Tissue Homeostasis, Institute for Stem Cell Biology and Regenerative Medicine, India
[2]Manipal Institute of Technology, India

npincha@gmail.com

Fibrosis is a pathological condition, which forms a common link between cancer, autoimmune disorders like scleroderma, myocardial infarction and numerous other diseases that involve the excessive deposition of connective tissue in an organ. Given its widespread occurrence, fibrosis contributes to one-third of deaths worldwide, making a study of the causal mechanisms of this condition imperative to identify therapeutics which are currently lacking. The hallmark of fibrosis is the excessive deposition of extracellular matrix components including collagen, which results from the activation of fibroblasts via different pathways. One of these pathways requires an immune cell type known as mast cells. Mast cells have been associated with increasing severity of the fibrotic phenotype in numerous disorders of the kidney, liver, lung as well as systemic sclerosis. In recent years, it has also been observed that apart from the mast cell secretome which comprises of fibroblast activating substances, a direct adhesion between these two cell types is also pre-requisite for fibroblast activation. However, the trigger for the increase in mast cell numbers in fibrotic tissue, as well as the mechanism(s) by which they adhere to fibroblasts and contribute to fibrogenesis, are questions which as yet remain ill-defined.

We addressed these open questions using a mouse model of skin fibrosis, wherein the transcription factor Snail is ectopically overexpressed in the epidermis of the skin. Our data reveals that the protein Plasminogen Activator Inhibitor type 1 (PAI-1), secreted from the Snail transgenic epidermal cells, impacts fibrogenesis at the inflammatory stage of disease development. Removal of PAI-1 in the Snail transgenic mouse skin results in a significant rescue of the fibrotic phenotype. Using this mouse model we have elucidated a novel role of PAI-1 as chemotactic factor to stimulate mast cell migration into the skin. Morever, we have discovered that it also facilitates mast cell adhesion to fibroblasts. Interestingly, we have observed a similar attachment between mast cells and fibroblasts in Scleroderma patient skin samples where the expression of PAI-1 in known to be high. Our observations suggest that the PAI-1 mediated upregulation of mast cell-fibroblast attachment results from an increase in the expression of a novel mast cell receptor on the fibroblasts via activation of the αV integrin-Focal Adhesion Kinase (FAK) signaling pathway. Furthermore, this heterotypic cell-cell adhesion culminates in the reciprocal activation of both the mast cells and fibroblasts. The resultant fibroblast activation then contributes to the development of skin fibrosis in the Snail transgenic mouse.

Thus, we have identified heretofore unknown mechanisms by which PAI-1 functions as an integral component in fibrogenesis. The role of mast cells in a pro-fibrotic context has so far been controversial, and our data strongly hints at the importance of mast cells in the development of various fibrotic diseases. We aim to identify the novel receptor for this mast cell adhesion on fibroblasts, which might function as a potential therapeutic target in the future.

The role of hair follicles in dermal remodelling and scar reduction

M. Plotczyk / CA. Higgins

Department of Bioengineering, Imperial College London, United Kingdom

magdalena.plotczyk16@imperial.ac.uk

Introduction

It has long been postulated that the hair follicle has a role facilitating cutaneous wound healing and reduction of scarring. In a recent clinical study grafting of scalp hair follicles into chronic venous leg ulcers significantly accelerated wound closure[1,2]. However, the mechanism by which the follicle dermis promotes wound remodelling remains unclear[3]. Many changes such as remodelling of extracellular matrix (ECM) and alterations in angiogenesis occur in the skin dermis during the hair growth cycle[4]. Interestingly, the same physiological events are observed in the final stages of wound healing, tissue formation and remodelling. To assess the mechanisms of dermal remodelling as a result of hair implantation, we have started a clinical study whereby hair follicles are transplanted into stretched scars on the scalp. The scars are all present in the same anatomical location and all result from lateral surgical incision of a skin strip for hair follicle transplantation. In our study, dermis is assessed in the un-remodelled scar, just prior to implantation, and 2, 4 and 6 months post implantation. To establish baseline conditions for the study, we are also studying un-remodelled dermis from established stretched scars on the occipital scalp, comparing it to healthy dermis in terms of structural, mechanical and cellular properties.

Results

Using Second Harmonic Generation (SHG) imaging we found a significant increase in collagen fibre alignment and thickness in occipital scalp scarred dermis compared to healthy dermis (Fig. 1). In addition, elastin fibres within the scar tissue dermis are being asessed with two photon microscopy. Using atomic force microscopy to characterise scalp scars versus healthy tissue we also found a varied stiffness distribution.

Perspectives

We hypothesize that the observed differences in ECM and dermal mechanical properties in scars put fibroblasts under variable strain, which in turn affects their behaviour in scarred dermis. Transcriptomic analysis will be used to detect differential gene expression between scarred and healthy dermis, and we will use this to identify pathways involved in cellular response to mechanical changes. The expression of selected candidate genes will be then tested during the progressive dermal remodelling induced by hair grafting. In the long-term we will exploit this understanding to design therapeutic strategies to reduce scarring.

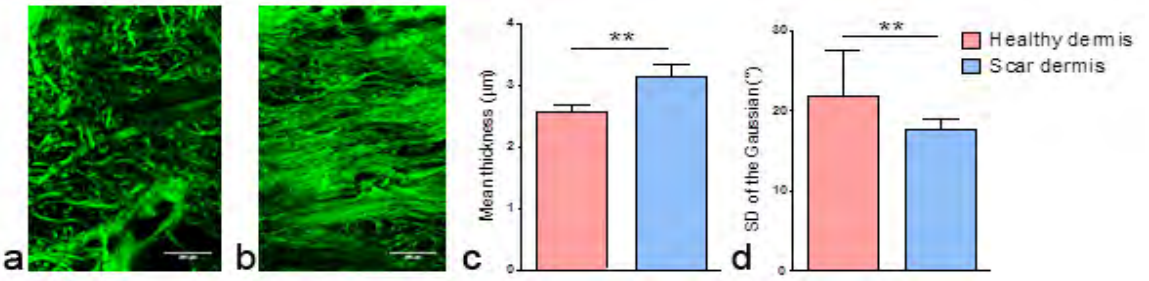


Fig 1. SHG imaging of collagen fibres in healthy remodelled dermis (a) and un-remodelled scarred dermis (b) Quantification of fibre thickness (c) and orientation angle dispersion (d) Mean ± SEM, t-test $p \leq 0.01$

References

1. Jimenez F., *et al*. Wound Repair Regen. 2012;20(6):806–14.
2. Martínez M.L., *et al*. Jaad. 2016;75(5):1007-1014.
3. Jimenez F, *et al.* Exp Dermatol. 2015;24(2):91–4.
4. Ansell D.M., *et al.* J Invest Dermatol. 2011;131(2):518–28.

Regulation of Macrophage Polarization by Fibroblasts

S. Saupe / R.A. Ferrer / M. Grünwedel / E. Wandel / A. Saalbach / S. Franz

Department of Dermatology, Allergology and Venerology, Leipzig University Medical Center, Leipzig, Germany

susann.saupe@medizin.uni-leipzig.de

A crucial checkpoint in the wound healing process is the resolution of inflammation. Essential for that is the switch in macrophage (Ma) activity from an inflammatory activated phenotype (M1) to an alternative pro-repair/resolving phenotype (M2). This polarization is regulated by the surrounding microenvironment. Several soluble factors, which inhibit the inflammatory activation of M1 or support the polarization toward M2, have been described including IL-10, transforming growth factor ß (TGFß), tumor necrosis factor-inducible gene 6 protein (TSG-6), or products of cyclooxygenase-2 (cox-2) such as prostaglandin E2 (PGE2) or D2 (PGD2).

Despite that, the mechanisms that control this M1/M2 switch during wound healing are not completely understood. Here we show that dermal fibroblasts (dFb) under the influence of the cytokines TNF, IL-1ß and GM-CSF which are typically present in inflamed tissue are capable to regulate Ma polarization via secretion of the immunomodulatory factors TSG-6 and cox-2 products. Thus, injection of dFb into mice with thioglycollate-induced peritonitis promotes the activation of alternative macrophages releasing high amounts of IL-10. Consequently, administration of dFb in wound margins improves defective tissue repair in db/db mice by reducing inflammation and favoring pro-repair macrophages. Based on these results we currently investigate whether dFb perform crucial immunoregulatory functions during dermal wound healing and further characterize the mechanisms how dFb induce the transition of inflammatory to alternative/pro-repair macrophage phenotypes.

We first analyzed whether the identified immunomodulatory factors are expressed by dFb during the course of wound healing using the full-thickness wound healing model in mice. Wounds were harvested at different time points post wounding (pw). Different cell types were sorted via FACS, RNA was extracted and then used for cDNA synthesis and q-PCR. We observed in the early phase of healing process (day 1 till day 3 pw) increased expression levels of TSG-6 in dFb fractions compared to other cell types.

We observed an additional peak of TSG-6 expression in dFb fraction starting at day 10 pw. These data correspond to the fact that TSG-6 is known to have anti-inflammatory and tissue reparative properties. As a member of the hyaluronan-binding protein family it interacts with hyaluronan (HA) and is involved in extracellular matrix remodeling. There are data describing that HA-rich ECMs interact with cells through receptors like CD44, which is also expressed on Ma.

So far there is no known receptor of TSG-6 but its recognition via CD44 has been suggested and thus might be a possible mechanism how TSG-6 mediates its immunomodulatory effect on Ma. Since dFb synthesize both TSG-6 and HA, we will further explore the role of HA and TSG-6 in the immunomodulatory crosstalk between dFb and Ma.

Sphingosine -1- phosphate receptor 5 modulates early stage processes of fibrogenesis in a mouse model of scleroderma: a pilot study

K. G. Schmidt[1] / M. Herrero San Juan[1] / S. Trautmann[2] / L. Berninger[3] / A. Schwiebs[1] / F. Ottenlinger[1] / D. Thomas[2] / F. Zaucke[3] / J. M. Pfeilschifter[1] / H. H. Radeke[1]

[1]Pharmazentrum Frankfurt/ZAFES / Inst. of Pharmacology and Toxicology, Hospital of the Goethe Univ., Frankfurt, Germany
[2]Pharmazentrum frankfurt/ZAFES / Inst. for Clinical Pharmacology, Hospital of the Goethe University Frankfurt, Germany
[3]Dr. Rolf M Schwiete, Research Unit for Osteoarthritis / Orthopaedic University Hospital Friedrichsheim gGmbH, Germany

K.Schmidt@med.uni-frankfurt.de

Introduction

Systemic sclerosis (SSc) is an autoimmune and connective tissue disease with unclear pathogenesis characterized by progressive skin fibrosis[1]. Crucial events during the early phase of disease are an exaggerated Th2 response and transforming growth factor β (TGF-β)- induced fibrogenic processes. Sphingosine-1-phosphate (S1P) is elevated in the sera of SSc patients and has been shown to affect above-mentioned processes depending on the targeted receptor[2-4]. In recent years it has been suggested that S1P receptor 5 (S1P5) contribute to fibrotic processes but to date S1P5 function has hardly been studied in the context of dermatofibrosis[5]. In this pilot study, we investigated the involvement of S1P5-mediated effects on early stages of dermal fibrogenesis in a low-dose bleomycin (BLM)- induced SSc mouse model.

Results

By comparing two-week BLM-treated skin areas (25 µg/day, 5 days/week) of wildtype (WT) and S1P5-deficient ($S1P5^{-/-}$) mice, we detected that S1P5 deficiency prevents the transcriptional increase of Th2-characteristic transcription factor *GATA-3* and *S1P receptor 3* under treatment-induced inflammatory conditions. Besides similar mRNA-induction of TGF-b1 (WT: 1.7 ± 0.2 fold; $S1P5^{-/-}$: 1.9 ± 0.4 fold), the transcriptional up-regulation of the downstream target *pro-collagen type 1* was only found in the WT mice (WT: 2.5 ± 0.5 fold; $S1P5^{-/-}$: 0.9 ± 0.3 fold). Additionally, the mRNA expression of the regulatory subunit *SMAD7* was S1P5-dependent regulated in the genotypes upon two weeks of BLM-injection. Nevertheless, a similar increase in dermal thickness was observed in the skin histology of WT and $S1P5^{-/-}$ mice after four weeks of BLM-treatment. At the same point in time, a gain in the proportion of the fibrotic cartilage oligomeric matrix protein within the dermis was detectable as a trend in the WT and significantly in the $S1P5^{-/-}$ mice.

Perspectives

In summary, we propose that S1P5 plays a role as novel modulator during the early phase of BLM-induced fibrogenesis in murine skin. This study is an initial step in understanding the role of S1P5-mediated effects during early stages of dermal fibrogenesis, which may encourage the ongoing search for new therapeutic options for the treatment of fibrotic skin diseases such as SSc.

References

1. Desbois, A.C. and P. Cacoub, Systemic sclerosis: An update in 2016. Autoimmun Rev, 2016. 15(5): p. 417-26.
2. Tokumura, A., et al., Elevated serum levels of arachidonoyl-lysophosphatidic acid and sphingosine 1-phosphate in systemic sclerosis. Int J Med Sci, 2009. 6(4): p. 168-76.
3. Radeke, H.H., et al., Overlapping signaling pathways of sphingosine 1-phosphate and TGF-beta in the murine Langerhans cell line XS52. J Immunol, 2005. 174(5): p. 2778-86.
4. Maceyka, M. and S. Spiegel, Sphingolipid metabolites in inflammatory disease. Nature, 2014. 510(7503): p. 58-67.
5. Shi, F., et al., Pleiotropic FTY720 Is a Specific and Potent Therapy for Hypertrophic Scars. J Invest Dermatol, 2017.

Loss of ***Ercc1*** in renal tubular epithelial cells results in degenerative kidney disease and renal fibrosis

G. Slaats[1,2] / F. Braun[1,2] / S. Brähler[1,2] / I. Henke[3] / L. Frech[1,2] / F. Seelemeyer[1,2] / T. Benzing[1,2] / A. Sterner-Kock[3] / C. Kurschat[1,2] / B. Schermer[1,2]

[1]Department II of Internal Medicine and Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany
[2]Cologne Excellence Cluster on Cellular Stress Responses in Aging Associated Diseases (CECAD), Cologne, Germany
[3]Center for Experimental Medicine, Medical Faculty, University of Cologne, Cologne, Germany

gisela.slaats@uk-koeln.de

Introduction

Renal fibrosis occurs in the normal aging process. In addition it is enhanced in chronic kidney diseases and a hallmark of various renal ciliopathies. Although there is some phenotypic and mechanistic overlap between ciliopathies and classical aging syndromes, we cannot simply classify ciliopathies as a premature aging disease. The ciliary defects in ciliopathies are indispensable. Accumulating DNA damage or disrupted DNA damage response (DDR) signaling could be important pathogenic factors in fibrogenesis. We aimed to *in vivo* confirm the concept of an important role of DDR in ciliopathies and gain new insights into fibrosis development.

Results

We generated *Ercc1*$^{fl/fl}$ ksp:cre mice to create a knockout in renal tubular epithelial cells, to study the effect of DNA damage accumulation in kidney cells specifically. These *Ercc1*$^{fl/fl}$ ksp:cre mice were born normally, but developed progressive renal failure and survive up to 26 weeks of age (median). The kidneys are small and display interstitial fibrosis accompanied by immune cell infiltrates. Analysis of unaffected 8 week old mice did not reveal an increased immune response. The kidney phenotypes are currently being analyzed in detail.

In parallel, we analyzed the presence of renal cilia in the DNA repair defective mouse line *Ercc1*$^{\delta/-}$, displaying a progeroid phenotype. Immunofluorescence staining of Ercc1$^{\delta/-}$ mice kidneys reveals reduced ciliation, which provides evidence for a dynamic DDR-cilia connection *in vivo*. Consistently, microscopy of mouse IMCD3 cells depleted for *Ercc1* revealed lower numbers of ciliated cells in 3D spheroids. These data suggest that loss of ciliation could be initiated by increased DDR signaling.

Perspectives

Our results imply that DDR signaling reduces ciliation and leads to renal fibrosis, both features of renal ciliopathies. The *Ercc1* mouse models provide further evidence for a role of DNA damage in the pathogenesis of renal aging-associated fibrosis. This might help us to find therapeutic strategies to delay the onset of end-stage renal disease by interfering with the initiating events.

The role of ***Hippo*** signalling in liver regeneration

E. Verboven / I. Moya / G. Halder

Centre for Cancer Biology, VIB-KULeuven, Belgium

elisabeth.verboven@kuleuven.vib.be

The Hippo pathway was discovered in *Drosophila* due to the spectacular overgrowth phenotype of hippo mutants. The pathway is conserved in mammals, where its activity is mediated by the transcriptional coactivators YAP and TAZ. Although hyperactivation of YAP and TAZ can similarly cause dramatic overgrowth of the liver and other organs in mice, the normal function of YAP and TAZ is still poorly understood.

By analyzing liver regeneration in the mouse, we found that YAP is transiently activated in hepatocytes in response to acute liver injury. Normal adult hepatocytes, however, have little active YAP and hepatocyte-specific deletion of *Yap* and *Taz* has no overt phenotype in adult mice. On the other hand, after toxic liver injury we observe persistent apoptosis and delayed repair in mice with liver-specific *Yap/Taz* deletion. These data show that YAP is specifically involved in the regenerative response in the liver. We are currently working to understand the role of YAP and TAZ in cell survival and cell plasticity.

Epigenetic function of the lysine specific demethylase LSD1 in hepatic stellate cell activation during liver fibrosis

L. Wang / Bastian Ulmer / X. Yu / L. Steinheuer / H. Eischeid / R. Buettner / M. Odenthal

Institute for Pathology, University Hospital of Cologne, Germany

lwang7@smail.uni-koeln.de

Background and aim

Liver fibrosis represents the final common pathway of different forms of chronic liver disease, independently if chronic inflammation is caused by viral infection, alcohol abuse or other factors. Activated Hepatic Stellate Cells (HSC) are central players of liver fibrogenesis. In response to chronic inflammatory processes they transdifferentiate into myofibroblasts, characterized by enhanced contractility and highly elevated production of extracellular matrix proteins (ECM).

Recent findings suggest that epigenetic histone modifications play an important role in triggering processes of liver fibrosis. The lysine specific histone 3 demethylase LSD1 is shown to be a central mediator involved in tumor progression of different cancer types, but its function in inflammatory and fibrotic processes is not yet known. Hereby, we aimed to study the function of LSD1 in cellular and molecular mechanisms of liver fibrosis.

Methods

We inhibited LSD1 function in myofibroblastically activated human HSC cells (LX-2) using the HCI-2509 compound that specifically blocks LSD1 protein-protein interaction and substrate binding. In addition, CRISPR/Cas mediated LSD1 knock-out HSC clones were generated.

The influence of LSD1 silencing on profibrogenic HSC activation was further studied by expression profiling using hybridisation microarrays. The microRNA (miRNA) pattern in response to LSD1 inhibition was measured by qPCR arrays. Furthermore, protein expression was analysed by protein mass spectrometry followed by comprehensive signaling pathway analysis and validated by immunoblotting and immunochemistry.

Results

LSD1 inhibition led to a pronounced change in HSC morphology, viability and expression profiles. Hence, in response to LSD1 inhibition, HSC showed a morphology resembling quiescent HSC with long extensions and lipid droplets. In addition, after LSD1 inhibition, cell growth arrest was associated with a pronounced expression change in factors involved in cell cycle progression and DNA synthesis. The antifibrotic miR-29, inhibiting ECM synthesis, was upregulated.

Further transcriptomic and proteomic studies revealed an altered expression profile of profibrogenic markers such as collagen subunits and smooth muscle actin. Most notably, LSD1 inhibition resulted in a distinct change in epigenetic pathways suggesting a LSD1 mediated control of downstream epigenetic targets such as histone deacetylases e.g. HDAC4 and the histone methyl transferase EZH2.

Conclusions

Our studies demonstrate that LSD1 plays a central role in liver fibrosis due to a change in the epigenetic control of gene expression. Ongoing chromatin immunoprecipitation (ChIP) and *in vivo* studies, using murine liver fibrosis models, will shed light on novel therapeutic options of LSD1 inhibition in chronic liver disease.

Transcriptomic meta-analysis of healing wounds, tumor stroma, wound-derived fibroblasts and cancer-associated fibroblasts identifies phase-specific gene signatures, which categorize tumor fibroblasts into wound-associated phenotypes

M. S. Wietecha / D. Lauenstein / S. Werner

Institute of Molecular Health Sciences, Department of Biology, ETH Zurich, Switzerland

mateusz.wietecha@biol.ethz.ch

Introduction

Though it has been long-recognized that wounds and tumors share phenotypic similarities, the cellular and molecular parallels between healing and tumorigenesis are still insufficiently characterized. Fibroblasts in the granulation tissue of wounds and in the stroma of tumors deposit and remodel the extracellular matrix (ECM), and they establish and maintain the essential microenvironments for healing and tumorigenesis. It has been shown that fibroblasts comprise a heterogeneous, alternatively-activated cell population in wounds and tumors, but in-depth investigations of molecular similarities and differences between wound-derived fibroblasts (WDFs) and cancer-associated fibroblasts (CAFs) are lacking.

Results

Using RNA-seq and microarray data available in the public repositories in addition to our own new sequencing data of FACS-sorted fibroblasts, we undertook a comprehensive meta-analysis comparing the transcriptomes of wounds and tumors in general, and the transcriptomes of WDFs and CAFs in particular. We first categorized genes associated with each phase of wound healing and found remarkable correlation in time-course gene expression of whole wounds and WDFs. We thus determined the molecular profiles of quiescent fibroblasts (normal tissue) and activated WDFs from all phases of repair: early-response (inflammatory), middle-response (proliferative, early ECM deposition/remodeling), late-response (late ECM deposition/remodeling). Next, we compared the transcriptomes of human and murine tumor stroma (skin, breast, prostate, ovarian) and CAFs (skin, oral, breast, lung, prostate, pancreas) to the wound healing signatures. Strikingly, we found that even though CAFs are highly heterogeneous across and within studies depending on tumor location and stage and patient-to-patient variability, they can be broadly categorized into wound phase-specific gene expression profiles found in WDFs, thus allowing for the prediction of their phenotype. Thus, we identified wound phase-specific gene subsets, which may discriminate CAFs into hypothesized alternatively-activated phenotypes. We also discovered a novel set of genes, which identifies most tested CAFs, i.e. candidate pan-CAF markers. In addition, we identified genes, which are expressed in whole wounds or in tumor stroma but not in fibroblasts, as well as genes, which are exclusively expressed/regulated either in repair or in tumor conditions. Recently published CAF proteomics studies validated candidate genes from our subsets at the protein level in cellular extracts, secretome and deposited ECM. Further integration of WDF and CAF data with published transcriptomes of cultured fibroblasts stimulated by serum, IL-1β, TGF-β and others revealed potential mechanisms of alternative activation of fibroblasts in wounds and tumors.

Perspectives

We present the first comprehensive meta-analysis of similarities and differences in gene expression between whole wounds and tumors, and more specifically between WDFs and CAFs. The results strongly suggest a possibility to phenotypically classify CAFs, tumor stroma, and even the stromal gene expression component of whole tumors based on reliable signatures of wound phase-associated genes. Such classification of pathological samples to the well-studied time-course of healing could inspire new diagnostics and therapies for combating carcinogenesis and improving healing.

Does the collagen-matrix provide a mechanical barrier for efficient entry of herpes simplex virus 1 into the dermis?

L. Wirtz[1] / D. Knebel-Mörsdorf[1,2]

[1]Center for Biochemistry, University of Cologne, Germany
[2]Department of Pediatrics and Adolescent Medicine, University Hospital Cologne, Germany

lisa.wirtz@uni-koeln.de

Dermal fibroblasts are natural target cells of the human pathogen herpes simplex virus 1 (HSV-1) upon wounding of skin or during recurrent infection. This is in line with our observation that primary murine and human dermal fibroblasts are susceptible for HSV-1.

While e*x vivo* infection of epidermal sheets results in efficient viral entry, no infected cells are observed after *ex vivo* infection of complete skin samples[1] suggesting that the apical surface of the epidermis as well as the dermis are protected against invasion of HSV-1. Here, we addressed whether HSV-1 can penetrate the dermal matrix to enter fibroblasts and initiate infection.

Infection studies were performed in 3D collagen cultures with primary murine dermal fibroblasts and increasing concentrations of collagen type I. Interestingly, we observed expression of viral transcripts in collagen-embedded fibroblasts. With increasing concentrations of collagen, however, infection was delayed. These observations were made in collagen-embedded fibroblasts under high as well as low mechanical tension. Furthermore, we *ex vivo* infected murine dermis of newborn back skin and adult tail skin. In dermis samples prepared from adult tail skin HSV-1 infection was even more delayed than in newborn dermis.

We also initiated *ex vivo* infection studies in human dermal sheets to analyze viral invasion. Preliminary results suggest that viral transcripts are only detectable at 24 h post infection.

Our results suggest that HSV-1 can reach its receptors on fibroblasts that are embedded in a collagen-matrix. In contrast to epidermal sheets, however, initiation of infection is strongly delayed suggesting that the collagen matrix acts as mechanical barrier for HSV-1.

References

1. Rahn E, Petermann P, Thier K, Bloch W, Morgner J, Wickström SA, and Knebel-Mörsdorf D. 2015. Invasion of herpes simplex virus type 1 into murine epidermis: an ex vivo infection study. J. Invest. Dermatol. 135, 3009-3016

Molecular basis of controlled ***in vivo*** cellular reprogramming that enables cardiac regeneration

W. Yao[1,2,3] / G. Ahuja [1,2,3] / S. Frank [1,2,3] / D. Bartsch [1,2,3]/ N. Russ [1,2,3] / C. Dieterich [4] / L. Kurian [1,2,3]

[1]Center for Molecular Medicine Cologne, Germany
[2]Institute for Neurophysiology, University of Cologne, Germany
[3]CECAD, University of Cologne, Germany
[4]University of Heidelberg, Germany

yao.wenjie@uk-koeln.de

Introduction

During embryogenesis the cellular fates remain restricted to keep the integrity and function of the tissue/ organ that it constitutes. Importantly, each cell type harbours a degree of plasticity which defines its ability for regenerative repair. Cardiac regeneration represents a natural and controlled reprogramming of cardiomyocytes to a pro-regenerative embryonic stage.

Remarkably, lower vertebrates such as zebrafish can regenerate the injured heart, in contrast to the mammalian counterparts which possess a limited regenerative capacity. However, our current knowledge on the mechanistic basis of cellular reprogramming that enables cardiac regeneration is still very limited.

Results

To bridge this apparent gap-of-knowledge, we first generated a high resolution, comprehensive temporal transcriptomic map of zebrafish cardiac regeneration, mouse and human cardiac development. We then developed a robust computational algorithm, which integrates the known regeneration regulators, expression dynamics, cell-type specificity, evolutionary conservation, and microRNA (miR)/ transcription factors (TF)/ RNA binding proteins (RBP)-target predictions, to identify major co-regulatory nodes.

Our bioinformatics analyses revealed two key interdependent molecular nodes mediating de-differentiation and re-differentiation of cardiomyocytes. Central to each of these nodes were a set of novel and uncharacterized microRNAs, which were predicted *in silico* to target the key RBPs, cardiac TFs and sarcomeric genes. Furthermore, using *in vivo* inhibitors and mimics for handful of these miRs, we found their potential role in cardiac development.

For example, mimics of the most promising candidate resulted in abnormal gastrulation and looping of the heart, even at lower concentrations. In addition, CRISPR/Cas9 mediated knockout of the predicted RBP target resulted in similar phenotypes as expected. Our preliminary evidence suggests that components of the novel pro-regenerative networks are integral to programming cardiac fate as predicted.

Perspectives

Encouraged by this, we will perform the exogenous manipulation of these RNA-regulons induce regenerative reprogramming of mammalian cardiomyocytes *in vitro* and *in vivo*. Finally, we aim to characterize the molecular mechanism of these RNA-regulons in cardiac regeneration. Ultimately, we intend to gain key fundamental molecular insights and as a first step to devise novel strategies to regenerate the mammalian heart.

Fibrillin-1 controls dermal fibrosis

A. Zuk[1] / C. Adamo[1] / Anja Niehoff[2]/ Wilhelm Bloch[2] / Jürgen Brinckmann[3] / Gerhard Sengle[1,4]

[1]Center for Biochemistry, Medical Faculty, University of Cologne
[2]German Sport University Cologne
[3]Institute of Virology and Cell Biology, University of Lübeck
[4]Center for Molecular Medicine Cologne, University of Cologne

cadamo1@uni-koeln.de

Introduction

Supramolecular networks composed of multi-domain extracellular matrix (ECM) proteins, such as fibrillin-1, represent intricate cellular microenvironments required to balance tissue homeostasis and direct remodeling. The importance of fibrillin-1 dependent networks is reflected by a number of multi-system disorders caused by human and murine fibrillin-1 mutations with often times opposing phenotypes. For instance, fibrillin-1 deficiency results in skin conditions raging from thick, stiff, and fibrotic to thin, hyperelastic and fragile skin.
350 kDa fibrillin-1 glycoprotein monomers assemble into beaded microfibrils representing the core scaffold onto which tropoelastin is deposited and crosslinked to give rise to the three-dimensional network of elastic fibers. As ubiquitously expressed and stretchable architectural entities, fibrillin microfibrils are not only essential for the integrity of tissues but also for defining their biomechanical properties. In addition to their structural function, fibrillin microfibrils are also known to target and sequester growth factors of the TGF-β superfamily within the ECM. Our current working hypothesis is that the structural integrity of FMF defines the activation status of ECM embedded growth factors, and that depending on which structural function is affected by the individual fibrillin-1 mutation, either increase or loss of growth factor activity may be the consequence. To gain further insight into this mechanism, the skin of a haploinsufficient mouse model (*Fbn1*$^{+/-}$), and a *Fbn1* knock-in mouse model where the C-terminal half of fibrillin-1 is truncated and fused to an eGFP-tag (*Fbn1*$^{+/GT8}$), were analyzed.

Results

We found that fibrillin-1 haploinsufficiency triggers a fibrotic phenotype in skin of *Fbn1*$^{+/-}$ mice indicated by elevated collagen content, increased skin stiffness in sensitive stretch tests, locally increased TGF-β activity and epidermal hyperproliferation. In contrast, *Fbn1*$^{+/GT8}$ mice showed no signs of fibrosis by histological analysis and no increase of TGF-β activity, however, pathologically altered collagen cross-linking characteristic for fibrotic conditions was identified. Collagen crosslinking defects in *Fbn1*$^{+/GT8}$ mice were associated with elevated lysyl hydroxylase expression. *Fbn1*$^{+/GT8}$ mice showed hyperelastic skin due to FMF and elastic fiber fragmentation while the elastic fiber network in *Fbn1*$^{+/-}$ remained intact.

Perspectives

Our data clearly suggest that the structural integrity of FMF is required to sequester TGF-β growth factors in the skin. Defined mutations of fibrillin-1 trigger different pathological responses with different consequences for the collagen and elastic fiber network. *Fbn1* haploinsufficiency leads to increased collagen production probably due to a reduction of TGF-β targeting sites while presence of mutant fibrillin-1 within the matrix triggers degradative processes but also alters inter- and intra-collagen bridging. By controlling collagen homeostasis via growth factor targeting fibrillin microfibrils influence the biomechanical properties of the skin which also decisively impacts dermal-epidermal crosstalk.

Heterogeneity of adipose tissue remodeling in rats with obesity induced by monosodium glutamate

N. Belemets[1] / O. Kuryk[1] / T. Falalyeyeva[1] / O. Sulaieva[2]

[1]Department of Fundamental Medicine, Taras Shevchenko National University, Ukraine
[2]Department of Pathology, Ukrainian Research and Practical Centre of Endocrine Surgery, Ukraine

oksana.sulaieva@gmail.com

Introduction

Obesity plays an important role in development of various pathologies including metabolic syndrome, insulin resistance, cardiovascular diseases, type II diabetes mellitus, cancer etc. As far as obesity is defined as an excess of adipose tissue, it is obvious that understanding the mechanisms of adipose tissue remodeling under obesity is a key for developing a new strategy of obesity and its complications prevention. However, adipose tissues are diverse: there are strong sex and site-dependent differences in adipocytes activity, secretory function, adaptive reactions and adipocyte-macrophage interplay that determines the risks of metabolic and inflammatory complications. The goal of this study was to define adipose tissue remodeling under experimental obesity depending on sex and location.

Methods

The model of neonatal monosodium glutamate (MSG) induced obesity was used for this experiment. 40 newborn Wistar rats that were subdivided into two groups, each group contained 10 males and 10 females. MSG was subcutaneously injected (4 mg/g) into the treated group, whereas the same volume of physiologic solution was injected into the control group at 2^{nd} , 4^{th}, 6^{th}, 8^{th}, 10^{th} days of birth. Rats were euthanized at 16^{th} week after birth. Visceral (VAT), subcutaneous (SAT), gonadal (GAT) adipose tissues were excised and evaluated. Body weight and length (from the nose to anus) were measured and BMI was calculated. To assess the distribution and morphological features of fat, Sudan IV staining was performed by using frozen sections.

Results

MSM-treated rats were heavier than control animals from neonate through all the experiment period. Their BMI was significantly higher than in control group ($p<0.05$) due to increased adiposity suggesting obesity development. All kinds of fat depot were increased in MSG-group comparing with control in sex-dependent manner. MSG-treated males demonstrated prevalence of visceral and gonadal fat accumulation ($p<0.05$) with increased size of adipocytes. Hypertrophy of adipocytes was associated with pro-inflammatory remodeling of VAT and GAT in males – close packing of sized adipocytes was associated with irregular pattern of vessels growth and increased number of macrophages and lymphocytes. Females demonstrated predominance of fat accumulation in subcutaneous depot. The pattern of fat accumulation in females was the following: SAT>VAT>GAT. The different mechanism of SAT remodeling in female rats was identified – through hyperplasia with increase of small adipocytes number. Loosely packed small adipocytes were accumulated predominantly along blood vessels and deep layer of dermis reflecting their origin from stromal vascular fraction of cells.

Conclusion

Thus, neonatal MGS-treatment leads to obesity with different fat accumulation in males and females. Visceral adiposity was associated with adipocytes hypertrophy, vascular remodeling and inflammation.

Perspectives

Understanding pro-inflammatory adipose tissue remodeling mechanisms is essential for developing the new strategy of treatment and prevention of metabolic and inflammatory complications of obesity.

Characterisation of joint macrophages in early and end stage osteoarthritis

J. Garcia / W. Wei / K. Wright / C. Mennan / S. Roberts / J. Richardson / G. J. V. M. van Osch / Y. Bastiaansen-Jenniskens

[1]ISTM, Keele University, Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, UK
[2]Department of Orthopaedics, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

john.garcia@rjah.nhs.uk

Introduction
Inflammation in the synovium is a feature of osteoarthritis (OA) that contributes to the general degradation of the joint by the secretion of pro-inflammatory molecules and degradative enzymes. The infrapatellar fat pad (IPFP) is an intra-capsular and extra-synovial adipose tissue that is also a source of pro-inflammatory molecules. Macrophages that reside in both the IPFP and synovium can exist in an M1 (pro-inflammatory) or M2 (ant-inflammatory) polarisation state. In this study, we have characterised the macrophage population in donor-matched IPFP and synovium, and assessed their modulation within the IPFP.

Results
Donor-matched IPFP and synovium tissues were obtained from patients undergoing cell therapy, debridement (early OA, n=7) or total knee replacement (end stage OA, n=4). Tissues were sectioned and immunohistochemistry was used to reveal the presence of cells that were positive for CD68 (pan macrophage marker), CD86 (M1), CD206 (M2) and arginase-1 (M2). IPFP explants were treated with the corticosteroid, triamcinolone, after which the tissues were digested to obtain the stromal fraction. Flow cytometry was then used to assess the phenotype of freshly isolated macrophages, using CD14 (pan macrophage marker), CD163 (M2), CD206 (M2), CD80 (M1) and CD86 (M1).

The IPFP and synovium were positive for both M1 and M2 macrophages markers. No significant difference was observed in the densities of macrophages (CD68+) found in the synovial intima, subintima and IPFP when comparing early and end stage OA patients. There was a strong correlation of CD68 positivity between the synovium and the stroma, but not between the synovium and IPFP or the stroma and IPFP. A similar trend was observed for CD206, but arginase-1 correlated positively between the synovium and FP. There was no significant difference in the percentage of macrophages in the stromal fraction of the synovium and IPFP of end stage OA patients for all the markers tested. More CD14+CD163+ cells were present in the stromal fraction of IPFP explants treated with triamcinolone (74.1%±5.5), compared to untreated controls (49.6%±2). CD14+CD86+ cells were moderately increased after treatment (82.2%±6.5) compared to controls (77.7%±2.5). A co-positivity of CD163 and CD86 was observed regardless of treatment.

Perspectives
Our data suggests that there is no correlation between the presence of macrophages in the IPFP and synovium, which could indicate that these tissues function differently with regard to macrophage prevalence and macrophage-associated inflammation. The co-expression of both pro- and anti-inflammatory markers in the synovial and IPFP tissues of end stage OA patients could be accounted for by the fact that macrophages can exist on a spectrum of phenotypes, as opposed to a strict M1/M2 polarisation. We have also demonstrated that the phenotype of macrophages can be modulated in situ towards an anti-inflammatory phenotype. These findings could have significant implications in understanding the role of macrophages in the progression of OA and in developing therapies to target macrophage related joint inflammation. However, a better understanding of the phenotypes of macrophages in the synovial joints is warranted.

Photodynamic therapy leads to time dependent regression of pathological corneal (lymph)angiogenesis and promotion of high-risk corneal graft survival

Y. Hou[1] / Viet Nhat Hung Le[1] / T. Clahsen1, A. C. Schneider[1] / Felix Bock[1,2] / C. Cursiefen[1,2]

[1]Department of Ophthalmology, University of Cologne, Germany
[2]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Germany

yhou@smail.uni-koeln.de

Purpose
Pathological corneal (lymph)angiogenesis is a known risk factor for immune-mediated allograft rejections after corneal transplantation (keratoplasty). However, there is no established treatment to regress pre-existing pathological corneal blood and lymphatic vessels in vascularized high-risk eyes prior to corneal transplantation. This study assessed the possibility to regress both vessel types by photodynamic therapy (PDT) after application of a photosensitizer, the influence of timing of PDT after application of the photosensitizer and the effect on graft survival in subsequent experimental murine high-risk keratoplasty.

Methods
Female BALB/c mice were used for inflammatory corneal neovascularization to induce combined hem- and lymphangiogenesis. Thereafter, the treated group received PDT in a series of time points after an application of photosensitizer (control group: phosphate buffered saline (PBS)). Eyes were excised for immunohistochemistry examine after PDT. Corneal flatmounts were stained to quantify hem- and lymphangiogenesis morphometrically using image analysis software. Graft survival proportion was compared between high-risk recipients with and without PDT via Kaplan–Meier survival curves.

Results
Corneal blood vessels showed a significant reduction when PDT was performed at early stage after photosensitizer application ($p<0.05$) while lymphatic vessels showed no significant difference. Both blood vessels (BV) and lymphatic vessels (LV) were significantly regressed when PDT was performed at the late stage after photosensitizer application (BV: $p<0.05$; LV: $p<0.01$). Long-term allograft survival increased significantly in PDT-treated eyes compared with controls ($p < 0.05$).

Conclusions
PDT after photosensitizer application can selectively regress pre-existing corneal blood vessels or both blood and lymphatic vessels depending on the timing of PDT. Performing PDT at the late stage destroys both vessel types. Pretreatment of recipients with PDT promotes graft survival in high-risk recipients. This PDT strategy may be a promising new method to reduce immune-mediated graft rejections and improve graft survival in high-risk eyes in the clinic.

StarPEG-heparin microgels – customised materials for promotion of wound healing

S. Kühn[1] / U. Freudenberg[1,2] / J. Thiele[1,3] / C. Werner[1,2]

[1]Leibniz Institute of Polymer Research Dresden, Max Bergmann Center of Biomaterials, Institute of Biofunctional Polymer Materials, Germany
[2]DFG-Center for Regenerative Therapies Dresden (CRTD), Technische Universität Dresden, Germany
[3]Leibniz Institute of Polymer Research Dresden, Institute of Physical Chemistry and Polymer Physics, Leibniz Research Cluster, Germany

kuehn-sebastian@ipfdd.de

Chronic wounds, a result of impaired wound healing, can cause significant morbidity and mortality. An imbalance of inflammatory and regenerative cytokines causes a wound state of self-sustained inflammation that impedes successful tissue regeneration (cell proliferation, matrix deposition, wound closure and angiogenesis). In recent studies wound healing was improved significantly either by scavenging of pro-inflammatory cytokines (e.g. MCP-1 and IL-8) or by controlled release of anti-inflammatory/pro-regenerative cytokines (e.g. IL-4, VEGF and TGFβ) from starPEG-heparin hydrogels[1-3]. A powerful approach to fully exploit the potential of heparin-based hydrogels and to promote the healing of chronic wounds most effectively would be to capture both, scavenging and delivery of relevant cytokines within one multifunctional customised wound dressing material. Therefore, defined starPEG-heparin microgels will be developed that can further be embedded into a hydrogel matrix and enable the locally and temporally controlled delivery of cytokines as well as the scavenging of pro-inflammatory cytokines within one multiphasic hydrogel material.

StarPEG-heparin gel matrices crosslinked by Michel type addition were processed into microgels using an established microfluidic method, i.e. flow focussing. The resulting microgels appeared highly monodisperse in size and confocal laser scanning microscopy revealed a homogeneous distribution of the material throughout the gels. The microfluidic setup could be adapted in order to tune the microgel size over a range of 25 - 70 µm through the adjustment of water to oil flow rates as well as the dimension of the microfluidic channel. Atomic force microscopy based nano-indentation measurements confirmed highly similar mechanical properties of the particles compared to the bulk reference material. Furthermore, varying the microgel composition allowed for the adjustment of mechanical properties within the range of 2 - 20 kPa. Overall, it was possible to synthesise well-defined microgels, with independent control over microgel size, mechanical and biochemical properties, that will lay the foundation for multiphasic hydrogel materials.

Future experiments involve the synthesis of microgels with selectively desulfated heparin as well as release and scavenging experiments with relevant cytokines. Previous work has clearly demonstrated how selective removal of sulfate groups can be utilised to alter the heparin affinity for cytokines and fine tune cytokine release from starPEG-heparin hydrogels[4]. Furthermore, microgels will be embedded into bulk gel matrices and the mechanical properties and cytokine loading of the multiphasic material will be customised to the requirements of wounded dermal tissue. Subsequently, the wound dressing will be assessed for its ability to simultaneously scavenge and deliver relevant cytokines *in-vitro* and *in-vivo* and to promote tissue regeneration in chronic wounds.

References

1. Lohmann N, Schirmer L, Atallah P, Wandel E, Ferrer RA, Werner C, et al. Glycosaminoglycan-based hydrogels capture Association for the Advancement of Science; 2017;9(386):eaai9044.
2. Schirmer L, Atallah P, Werner C, Freudenberg U. StarPEG-Heparin Hydrogels to Protect and Sustainably Deliver IL-4. Advanced healthcare materials. Wiley Online Library; 2016;5(24):3157–64.
3. Freudenberg U, Liang Y, Kiick KL, Werner C. Glycosaminoglycan-Based Biohybrid Hydrogels: A Sweet and Smart Choice for Multifunctional Biomaterials. Advanced Materials. Wiley Online Library; 2016.
4. Zieris A, Dockhorn R, Rơhrich A, Zimmermann R, Mu̇ller M, Welzel PB, et al. Biohybrid networks of selectively desulfated glycosaminoglycans for tunable growth factor delivery. Biomacromolecules. ACS Publications; 2014;15(12):4439–46.

Comprehensive identification of wound healing and inflammation miRNAs reveals a key role for miR-223 in neutrophilic clearance of ***Staphylococcus aureus*** at wound sites

R. Mori

Department of Pathology, Nagasaki University School of Medicine, Japan

ryoichi@nagasaki-u.ac.jp

Inflammation at a wound site is essential for preventing infection but misregulated inflammation leads to pathologies of healing including chronic non-healing wounds and scarring. MicroRNAs (miRNAs) are key regulators of the inflammatory response, acting by translational processing of target mRNAs. In the final step of miRNA processing, Argonaute 2 (Ago2)-bound mature miRNA (Ago2–miRNA) complexes bind to target mRNAs and inhibit their translation.

Using a novel immunoprecipitation purification system, we identify wound-induced Ago2-miRNA complexes during the murine repair process to generate a library for next generation sequencing. Of these wound upregulated miRs we identified four that are wound inflammation-related miRNAs (*miR-139-5p*, *miR-142-3p*, *miR-142-5p*, and *miR-223*). Skin wound healing in *miR-223* knockout (KO) mice was delayed concomitant with altered neutrophil activation.

We show that *miR-223* KO mice exhibit a prolonged acute inflammatory responses at aseptic wound sites and this is associated with neutrophil expression of interleukin-6, a *miR-223* target gene, as well as increased neutrophilic hyperoxidative bursts. In a typical clinical skin healing scenario, *Staphylococcus aureus* (*S. aureus*) is often a pathobiont and associated with chronic wound exacerbation. Intriguingly, *S. aureus*-infected *miR-223* KO mice showed markedly faster skin wound healing than infected WT mice; moreover, cell transplantation therapy using *miR-223* KO-derived neutrophils and *miR-223* antisense oligodeoxynucleotides knockdown in wounds, both markedly improved the healing of infected WT wounds.

This study demonstrates a key role for *miR-223* in regulation of the bacteriacidal capacity of neutrophils at wound sites, and since we show *miR-223* is upregulated in human inflamed skin samples, suggests that targeting *miR-223* might be of therapeutic benefit for enhancing the healing of infected wounds in the clinic also.

Modulating NF-κB signaling in the early wound as a means to accelerate tissue repair

R. Schuster / Y. Lior / T. Fadel / E. C. Lewis

Department of Clinical Biochemistry and Pharmacology, Faculty of Health Sciences,
Ben Gurion University of the Negev, Israel

ronensc@post.bgu.ac.il

Introduction

Proper wound healing hinges on a well-coordinated early inflammatory burst for priming local cell proliferation and active tissue remodeling. Conversely, dysregulated inflammation is a hallmark feature of chronic wounds as well as fibrotic lesions. Current wound treatment strategies focus on supportive care, infection prevention and infection control with few available therapies demonstrating efficacy in shifting a non-healing wound towards a healing state. Human α1-antitrypsin is a serum glycoprotein with potent anti-inflammatory and tissue protective properties. Unlike conventional anti-inflammatory therapies, hAAT has been shown to increase IL-1Ra, IL-10 and VEGF levels. Previous studies implicate the NF-κB pathway in this phenomenon yet the molecular mechanism is not fully understood. Genetic hAAT deficiency manifests as emphysema, hepatic damage and skin disturbances. Several clinical trials are testing hAAT therapy in non-deficient individuals for various conditions including GvHD, acute myocardial infarction and early onset type 1 diabetes. A shared feature of these conditions is unresolved tissue damage. However, few studies directly address the role of hAAT in tissue repair and its effect on the inflammatory response during wound healing. ***Aim:*** to examine the effect of hAAT treatment on NF-κB signaling in the early wound and its impact on subsequent wound healing rate.

Methods

Epithelial monolayers were mechanically wounded in the presence and absence of hAAT. NF-κB signaling dynamic and transduction was assessed under hAAT enriched conditions was assessed in epithelial and macrophage cell cultures. Cutaneous dorsal wounds in mice were administered topical or systemic hAAT.

Results

48 hours following wounding, hAAT treated epithelial monolayers achieved 69.64±0.57% wound repair compared to 24.13±0.92% in untreated controls. Pretreatment with p65 inhibitor JSH23 reduced monolayer repair rate to 33.31±1.71 in hAAT treated monolayers. Furthermore, 30 minutes following mechanical wounding, hAAT treatment increased NF-κB reporter signal by 3.57±0.077 fold compared to untreated controls. hAAT treatment modified cellular distribution and ubiquitination pattern of NF-κB subunit p65 in macrophage *in-vitro*. hAAT pre-treatment increased LPS induced IL-1Ra secretion increased to 66.71±1.99 ng/ml from 43.95±1.65 ng/ml in macrophage cultures. Addition of JSH23 abolished hAAT induced increase in IL-1Ra. Finally, topical administration of hAAT reduced dorsal cutaneous wound closure time from 9.6±0.62 days to 5.33±0.42 days.

Perspectives

Despite being an anti-inflammatory molecule, hAAT is able to expedite wound healing, an *inflammation* driven process. Indeed, we have found that AAT modulates but does not inhibit NF-κB signaling in macrophages and epithelial cells in-vitro. Moreover, treating epithelial cells with a NF-κB subunit p65 inhibitor (JSH-23) abolishes AAT induced epithelial monolayer repair. Whether or not AAT requires uninterrupted NF-κB signaling in order to expedite wound repair in-vivo is currently under investigation.

Keywords: Human α1-antitrypsin (hAAT), inflammation, wound healing, NF-κB, macrophage.

Identification of specific microRNAs in diabetic-derived neutrophils: functional analysis of miR-129-2-3p and inflammation-related genes

T. Umehara[1] / T. Murase[1] / R. Mori[2] / Y. Abe[1] / T. Yamamoto[1] / K. Ikematsu[1]

[1]Division of Forensic Pathology and Science, Unit of Social Medicine, Course of Medical and Dental Sciences, Graduate School of Biomedical Sciences, Nagasaki University School of Medicine, Japan
[2]Department of Pathology, Nagasaki University School of Medicine, Japan

umehara@nagasaki-u.ac.jp

Introduction

We have previously shown that the retention of neutrophils in diabetic cutaneous wounds is aberrant in mice, inflammation is prolonged, and the pathogenesis may be due to dysfunction of diabetic-derived neutrophils. MicroRNAs (miRNAs) are critical regulator for normal inflammatory response. Accordingly, in order to clarify the molecular mechanism of regulation of inflammation in diabetic-derived neutrophils, we identified specific miRNAs in these cells using microarrays and analyzed the function of miRNAs in diabetic-derived neutrophils.

Methods

Neutrophils from pooled BM of 3 db or 3 non-db mice were isolated using the neutrophil isolation kit (MACS), and miRNA was purified by MicroRNA isolation kit, Mouse Ago2 (Wako). Microarray analysis was performed on a total of 8 pools (4 pools of 3 db BM samples and 4 pools of 3 non-db BM samples) using SurePrint G3 Mouse miRNA microarray (Agilent Technologies). Luciferase reporter assay was performed using Dual-Glo luciferase assay system (Promega).

Results

Microarray analysis showed that diabetic-derived neutrophils expressed 26 miRNAs (*miR-144 family*, *miR-451a* etc.) signficantly higher than non-diabetic-derived neutrophils, and 82 miRNAs (*miR-129-2-3p*, *miR-149-3p* etc.) showed a significant decrease.

There were reported that these miRNAs were associated with cell proliferation, apoptosis, migration and invasion in some type of cancer. These miRNAs also might be involved in cell proliferation, migration and apoptosis in diabeteic-derived neutrophils. Luciferase reporter assay indicated that *miR-129-2-3p* directly regulated *Casp6*, *Ccr2* and *Dedd2* translation. These genes are involved in biological processes such as inflammatory response, apoptosis and phagocytosis.

Conclusions

These results suggest that *miR-129-2-3p* may be involved in the dysfunction of diabetic-derived neutrophils, and retention kinetics of neutrophil and chronic inflammation may be initiated by *miR-129-2-3p*-regulated genes.

Impact of depletion of microglia/macrophages on regeneration after spinal cord injury

M. Vulović[1] / M. Aksić[2] / I. Jakovčevski[3]

[1]Department of Anatomy and Forensic Medicine, Faculty of Medical Sciences, University of Kragujevac, Serbia
[2]Institute for Anatomy, Medical Faculty, Belgrade, Serbia
[3]Institute for Molecular and Behavioral Neuroscience, University Hospital Cologne, Center for Molecular Medicine Cologne, and Experimental Neurophysiology, German Center for Neurodegenerative Diseases, Bonn, Germany

igor.jakovcevski@uk-koeln.de

Introduction

Microglia/macrophages play important functional roles in regeneration after injury to the central nervous system. Infiltration of circulating macrophages and proliferation of resident microglia occur over minutes following spinal cord injury. The activation of microglia/macrophages clears tissue debris, but excessive activation may lead to excessive damage and hamper repair.

Results

To assess the role of microglia/macrophages in regeneration after spinal cord injury we used CD11b-HSVTK transgenic mice, in which viral thymidine kinase activates ganciclovir toxicity in CD11b-expressing myeloid cells, including macrophages and microglia. Ganciclovir was applied to the lumbar spinal cord using Alzet osmotic pumps during 4 weeks: 2 weeks before and 2 weeks after lower thoracic spinal cord injury to CD11b-HSVTK transgenic and wild-type control mice. We accomplished a 50% reduction in the number of microglia/macrophages at 1 week, and 15% at 6 weeks after injury. Next we evaluated motor recovery weekly during 6 weeks after injury. According to both the Basso mouse scale score and single frame motion analysis, the CD11b-HSVTK mice showed similar rate of motor recovery in comparison to control mice during the whole observation period, with the exception of the slightly better locomotor function in CD11b-HSVTK mice 1 week after injury. The catecholaminergic and cholinergic reinnervation of the spinal cord caudal to injury site 6 weeks after injury was similar between the CD11b-HSVTK and control mice. Astrocyte scaring was increased both at 1 and 6 weeks after injury in CD11b-HSVTK mice compared with controls.

Perspectives

A drastic reduction in microglia/macrophage population does not influence functional recovery and axonal regeneration after spinal cord injury, although it increases astrocyte scar formation.

Novel insights in the link between type 2 innate signals and initiation of pro-fibrotic pathways

S. Willenborg[1] / J. Knipper[1] / J. Brinckmann[2] / W. Bloch[3] / R. Wagener[4] / A. Niehoff[5] / S. Eming[1]

[1]Department of Dermatology, University of Cologne, Germany
[2]Department of Dermatology, University of Lübeck, Germany
[3]Department of Molecular and Cellular Sport Medicine, German Sport University Cologne, Germany
[4]Department of Biochemistry, University of Cologne, Germany
[5]Institute of Biomechanics & Orthopedics, German Sport University Cologne, Germany

sebastian.willenborg@uk-koeln.de

Introduction

Activation of the immune response is a critical early event during injury that determines the outcome of tissue restoration towards regeneration or replacement of the damaged tissue with a scar. The mechanisms by which immune signals control these fundamentally different regenerative pathways are largely unknown.

Results

In this study we have demonstrated that during skin repair in mice interleukin-4 receptor α (IL-4Rα)-dependent macrophage activation controlled collagen fibril assembly, and that this process was important for effective repair while having adverse pro-fibrotic effects. We could show that in mice with myeloid cell-restricted IL-4Rα-deficiency ($Il4ra^{MKO}$) skin repair was associated with delayed wound closure, massive hemorrhages in the granulation tissue, and disturbances in extracellular matrix architecture. Ultrastructural analysis of wound tissue in $Il4ra^{MKO}$ mice revealed an abnormal collagen fibril assembly.

Intriguingly, HPLC-based analysis of the granulation tissue revealed an altered collagen cross-link pattern when compared to control mice. Whereas granulation tissue in control mice was characterized by dihydroxy lysinonorleucine (DHLNL) collagen cross-links, a typical feature of fibrotic tissue, these crosslinks were significantly reduced in $Il4ra^{MKO}$ mice. To identify macrophage-derived mediators that control the formation of extracellular matrix architecture, we analyzed flow cytometry sorted wound macrophages. Interestingly, wound macrophages in $Il4ra^{MKO}$ mice revealed significantly reduced expression of Relm-α, a small cysteine-rich secreted molecule that is a hallmark of alternatively activated macrophages and has been associated with experimental fibrosis and pro-fibrotic conditions in human diseases. By using an in vitro macrophage-fibroblast co-culture system we identified Relm-α released from macrophages as inducer of lysyl hydroxylase 2 (LH2) expression in fibroblasts. LH2 is known to play a pivotal role directing DHLNL collagen cross-links. To substantiate a direct role of Relm-α in skin repair we characterized the wound healing response in Relm-α deficient ($Retnla^{-/-}$) mice.

Notably, we detected intriguing parallels regarding morphological, structural and biochemical alterations of the wound healing response in $Retnla^{-/-}$ and $Il4ra^{MKO}$ mice. As such the repair response in $Retnla^{-/-}$ mice was characterized by a highly hemorrhagic granulation tissue, transient delay in wound epithelialization, and altered assembly and size of collagen fibrils. Most importantly, HPLC-based analysis revealed reduced DHLNL collagen cross-links in $Retnla^{-/-}$ mice when compared to controls, and this was associated with reduced expression of LH2 in complete wound tissue as analyzed by qRT-PCR analysis. Importantly, local application of recombinant Relm-α restored LH2 expression and rescued disturbed granulation tissue formation in $Il4ra^{MKO}$ mice.

Perspectives

Collectively, our findings provide novel mechanistic insights in the link between type 2 immunity and initiation of pro-fibrotic pathways. Based on our findings novel anti-fibrotic therapeutic strategies to treat patients suffering from fibrotic disorders such as lipodermatosclerosis or systemic sclerosis can be developed.

Notes

Antisense morpholino oligonucleotides enter dystrophic muscle fibres via myoblast cell fusion ***in vivo*** and provide a rational approach for efficient dystrophin restoration in Duchene Muscular Dystrophy

U. Akpulat[1,2,3] / J. Novak[4] / K. Becker[1,2] / T. A. Partridge[4] / S. Cirak[1,2]

[1]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Germany
[2]University Hospital Cologne, Department of Pediatrics, Cologne, Germany
[3]Dept. of Medical Biology, Faculty of Medicine, Kastamonu University, Turkey
[4]Children's National Health System, Children's Research Institute, Center for Genetic Medicine Research, Washington DC, USA

sebahattin.cirak@uk-koeln.de

Duchenne muscular dystrophy (DMD) is an X-linked recessive lethal muscle-wasting disease that affects 1/3,500 males and is caused by out-of-frame mutations in the dystrophin gene. Milder phenotype of allelic disease, Becker muscular dystrophy (BMD) caused by in-frame mutations has served as model for exon skipping strategy as a promising therapeutic attempt for DMD patients. Morpholino antisense oligonucleotides (PMO-AO) have been employed to exclude disruptive exons from the mutant DMD transcript has elicited the restoration of truncated dystrophin, but partly functional protein in analogy to BMD, in dystrophic muscle.

Recently, a 30-nucleotide PMO for exon 51 skipping called Eteplirsen was conditionally approved by the FDA. Although Eteplirsen is suitable for 14% of all DMD patients. Its clinical beneficial effect for restoring dystrophin protein in DMD patients remains debatable. Systemic delivery of Eteplirsen to DMD patients has shown a dose dependant response, better for higher doses but with high variability and patchy dystrophin expression (Cirak et al., 2011). To identify the factors leading to this variability, we investigated the influence of myofibre regeneration on exon skipping by treating dystrophin-null mdx mice with a high-dose of morpholino together with timed pulses of bromodeoxyuridine. This enabled us to define the stage of regeneration, relative to systemic drug delivery, that coincides with optimal PMO uptake and exon skipping. Robust PMO uptake and efficient elicitation of dystrophin expression was localized to lesions regenerating during the 3 days prior to, or coincident with, PMO administration. PMO accumulation was exclusive to inflamed regions where it entered inflammatory cells, differentiating myoblasts and newly forming myotubes. We conclude much of the variability in PMO-induced dystrophin expression reflects the limitations on delivery imposed by the need for two concomitant events: first, release of PMO from the vascular bed into the interstitium by inflammatory exudation associated with myopathic lesions; second, fusion of PMO-loaded differentiated myoblasts into the newly repairing segments of muscle fibres (Novak et al., 2017).

Clinical trials proofed that the PMO backbone safe for systemic delivery in men but using higher doses of Eteplirsen could not be administered due excessive economic costs. To overcome this issue, one strategy is the reducing length of PMO, which would make them more economic. We used shorter 25-mer PMOs targeting same ESE exon 51 site as Eteplirsen on pre-mRNA to compare their efficiencies on Dystrophin-null exon 52 deleted H2K-mdx52 mouse myoblast cell line. All of three shorter PMOs and Eteplirsen has shown dose dependent response, while skipped-dystrophin induction was similar in amount at higher doses only one of then was comparable with Eteplirsen at lower doses. We validated dystrophin protein induction *in-vivo* by intramuscular injection of all shorter PMOs in the tibialis anterior muscle of the mdx52 mice.

Our findings are of importance to improve PMO delivery and reduce variability for exon skipping mediated therapies, especially for DMD and other genetic primary muscle diseases. Shorter PMO antisense oligonucleotides would allow higher doses at the same economic cost and would induce high level of dystrophin restoration in DMD muscle and ultimately increase the clinical efficacy.

Literature: Cirak et al., 2011, The Lancet and Novak et al., 2017 Nature Communication

Tailored sulfation patterns of heparin in biohybrid hydrogels for modulating cytokine signals by wound dressings

P. Atallah[1] / L. Schirmer[1] / U. Freudenberg[1] / C. Werner[1,2]

[1]Max Bergmann Center of Biomaterials, Leibniz Institut für Polymerforschung, Dresden, Germany
[2]Center for Regenerative Therapies Dresden, Technische Universität Dresden, Dresden, Germany

atallah@ipfdd.de

Glycosaminoglycan (GAG)-based hydrogels have gained growing interest in the field of tissue engineering due to their ability to emulate the natural interaction between the extracellular matrix and soluble signal mediators. The underlying electrostatic interactions between the sulfated, negative charged GAG, such as heparin, and various signaling molecules have been previously employed for the administration or sequestration of growth factors to modulate cell fate decisions. Although such strategies have been successfully utilized in different biomedical applications in the past, they are limited by the lack of control over the GAG – signaling mediator interaction and therefore the tunability of the mediator release from the scaffold. In here, we explore the modulation of the sulfation degree of the GAG as a means to better control the affinity between signaling mediators and the GAG.

Heparin was modified by selective desulfation procedures to yield chemically defined derivatives with variable sulfation patterns. The synthesized GAG building blocks with controlled sulfation were cross-linked with starPEG and various functional peptide units to form hydrogel networks of tunable bimolecular composition. Gradual desulfation of heparin within the starPEG-GAG hydrogels resulted in a tunable release of VEGF-165, a key player in angiogenesis and wound healing. Moreover, hydrogels made of desulfated heparin resulted in a more pronounced proangiogenic response in wound angiogenesis studies with in db/db mice *in vivo*[1].

In addition to the tunability of signaling mediator release from the scaffolds, the selective desulfation also allowed for the control over the protein sequestration profiles of starPEG-GAG hydrogels. This is especially important in complex signaling environments, such as wound healing, where a plethora of signaling mediators is involved. In particular, we were able to fine-tune the scavenging profile of the hydrogels for selectively binding and neutralizing inflammatory chemokines, but not to interact with pro-regenerative growth factors: starPEG-GAG hydrogels with defined sulfation patterns of the GAGs were shown to sequester chemokines with high capacity, leading to diminished immune cell influx, reduced inflammation and enhanced wound closure in an impaired wound healing mouse model[2].

Thus, different strategies based on starPEG-GAG hydrogels were successfully applied to modulate the cytokine levels in wounds to promote wound healing. The GAG sulfation pattern was shown to be a decisive parameter for tailoring the protein affinity of the GAG-based biomaterials to meet the requirements of specific applications.

References

1. U. Freudenberg, A. Zieris, K. Chwalek, M. V. Tsurkan, M. F. Maitz, P. Atallah, K. R. Levental, S. A. Eming, C. Werner, J. Control. Release 2015, 220, 79.

2. N. Lohmann, L. Schirmer, P. Atallah, E. Wandel, R. A. Ferrer, C. Werner, J. C. Simon, S. Franz, U. Freudenberg, Sci. Transl. Med. 2017, 9.

Vascular endothelium dysfunction in the patients with solid malignancies and their regression following the radical surgery

E. V. Cherepkova[1] / K. G. Gurevich[2] / A. E. Gorbatenko[3] / S. T. Olgaev[4] / I. G. Sheremetyeva[5] / I. A. Gribacheva[6] / K. P. Petrova[6]

[1] Budgetary State-Research Institute of Physiology and Basic Medicine, Russia
[2] Moscow State University of Medicine and Dentistry, Russia
[3] Semey State Medical University, Kazakhstan
[4] Almaty Regional Oncology Center, Kazakhstan
[5] Altai State Medical University, Russia
[6] Novosibirsk State Medical University, Russia

india06@mail.ru

Introduction

Endothelial dysfunction is closely associated with the disorders of vascular and platelet links of the hemostatic system and can be one of the causes of the thromboembolic complications in the patients with malignancies. However, its development can also be linked to the cardiovascular, lung and bronchi, renal, or gastro-intestinal comorbidities, frequently occurring the oncological patients.

The aim of this study was to evaluate the presence of functional dysfunction of vascular endothelium in the oncological patients and its dynamics in the course of radical surgery.

Materials and Methods

We studied 295 patients with gastric (adenocarcinoma, stage IB-IIIB), pancreatic (adenocarcinoma of pancreas head, stage IB-IIB), and hepatic (hepatocellular carcinoma, stage I-IIIA) malignancies. In all patients (age 41-75, mean age 62,8±4,3), the radical surgical treatment was performed with the specific antitumor therapies (radio- or chemotherapy) according to the clinical indications.

The content of desquamated endotheliocytes, the concentration of von Willebrand factor, and the level of endothelium-dependent vasodilation were determined before, 3 days, and 12 weeks after the surgery. Same parameters were determined in a control group of 85 clinically healthy subjects (age 40-68, mean age 55,4±2,7). The comparison of parameters between study and control groups was made using Mann-Whitney test, the dynamic comparison was performed using Wilcoxon test.

Results

In the pre-surgery period, the median content of circulating endotheliocytes and the concentration of von Willebrand factor were significantly higher (3.15 fold higher, $p<0.001$ and 1.67-fold higher, $p=0.018$, respectively) in the patient study group, as compared to the control group. A relative decrease of the level of endothelium-dependent vasodilation (1.45-fold, $p=0.007$) was also observed in the patient group. The most pronounced differences in all parameters under study were observed in early post-surgery period when the content of circulating endotheliocytes and the concentration of von Willebrand factor were 4.22-fold ($p<0,001$) and 1.88-fold ($p=0,004$) higher in the patient group, as compared to the controls. The level of endothelium-dependent vasodilation decreased, accordingly, by 1.63-fold ($p=0.003$) in the patients, as compared to the controls. These changes reflect the post-surgical lesions and overall stress to the body organs and systems. However, in a later post-surgery period we observed the tendency of return of endothelial parameters under study to their normal values. Twelve weeks post-surgery, the content of circulating endotheliocytes demonstrated only a 1.18-fold ($p=0.043$) increase over the control values. The difference in the concentration of Willebrand factor was also insignificant. The difference in the level of endothelium-dependent vasodilation has also virtually vanished (1.09-fold decrease, $p>0.05$)

Perspectives

Endothelial dysfunction in the oncological patients are associated with the presence of the malignancy, and they undergo a considerable regression following the radical surgery.

Retinal tissue engineering in the dish: from disease mechanisms to personalized regenerative medicine

E. Gabriel[1] / F. Schinzel[1] / M. Gottardo[1] / A. Ramani[1] / W. Albanna[4] / S.M. Sertel[2] / G. Bao[2, 3] / T. Schneider[4] / J. Gopalakrishnan[1]

[1]Laboratory for Centrosome & Cytoskeleton Biology, Center for Molecular Medicine Cologne, University of Cologne, Germany
[2]Institute of Neurophysiology and Cellular Biophysics, University of Göttingen, Göttingen, Germany
[3]DFG Research Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), University of Göttingen, Göttingen, Germany
[4]Institute of Neurophysiology, University of Cologne, Germany

egabriel@uni-koeln.de

Introduction

An important upcoming branch of regenerative medicine is human induced pluripotent stem cell (iPSC)-based tissue engineering. It opens up new possibilities to generate specific cell types and even tissues from the patient´s own material and therefore to

- Model the disease, for example the natural mutation of a genetic disorder, in the dish for studying the disease mechanism and drug testing
- To generate patient´s derived tissues for replacement therapy with lower risk of immune rejection.

Retinitis pigmentosa (RP) is a neuroretinal degenerative disorder due to insidiously progressive loss of retinal function. Patients carrying a genetic risk for RP lose their power of vision within decades. Maintenance of patients´ retinal function as long as possible by protecting photoreceptors from cell death is one of the therapeutic strategies. For this, it is crucial to shed light on and understand the underlying pathogenic processes in RP disease progression, for example by using animal models or *in vitro* models of RP. When maintenance and repair are no more possible, replacement of degenerated tissues by transplantation of *in vitro* engineered cells might be a therapeutic approach to restore patients´ vision.

Today, novel techniques of cell culture allow not only differentiation of homogenous cell monolayers but even growing of 3D tissue-like structures with self-assembled heterogeneous stratified cell layers. This is important as it allows understanding the cell-to-cell communication in a complex environment *in vitro*.

Here, we developed a method for the generation of human iPS cell derived brain organoids with eyecups, so-called eye organoids, which could serve both aspects for RP therapy. These eye-like structures developed from Pax6/Sox2 positive forebrain regions of 3D human brain organoids contain different relevant retinal cell types such as pigmented RPE cells and functional photoreceptors.

Results

We developed a new protocol for the rapid and robust generation of eye organoids from human iPS cells. This helped us to model early human eye development in the dish. Eye organoids contained ciliated RPE cells with a hexagonal morphology situated nearby photoreceptors. The photoreceptors were Rhodopsin/RAX positive and showed light response. Next, we will generate eye organoids from patients with Retinitis pigmentosa to

- model the retinal degeneration in the dish for studying the underlying mechanisms and
- use them for an *in vitro* therapeutic screening.

Perspectives

Eye organoids serve as a relevant tool to study human eye development in health and disease in a complex 3D tissue-like context. In future, we could dissect specific cell types from these artificial retinal tissues for transplantation of patient´s own cells to replace degenerated tissue areas. As perspective, 3D tissue engineering from human iPS cells will turn into a major branch of regenerative medicine for the disease modeling in the dish and for tissue replacement therapy.

Wound healing is cured by magnetic photons detected in homeopathic remedies

K. Lenger

Institute for Scientific Homeopathy, Offenbach, Germany

dr.karin.lenger@t-online.de

Homeopathy can now be explained by biochemical and biophysical laws, since Lenger discovered magnetic photons in highly potentized homeopathic remedies by two magnetic resonance methods. Homeopathy is efficious by the principle of magnetic resonance.

The homeopathic law of similars can be explained in that way: the frequency of the remedy must match the frequency of the patient. By applying highly potentized, mostly in 50M-potency levels, substrates, enzymes and inhibitors of the pathological pathways can be cured, the corresponding lab-values become normal. Wound healing and even inflammation of the veins need nearly the same homeopathic remedies. I developed some reaction chaines: Severe inflammation with pus is cured by the following highly potentized remedies: Anthracinum, Pyrogenium, the snake venoms of *Elaps corallines, Crotalus horridus, Lachesis mutus*, then Zincum metallicum, Arnica and Phosphorus. The snake venoms are used very often for healing the inflammations of the veins.

Moderate inflammation is healed by the following remedies: Kaliumbichromicum, Mercurius solubilis, Silicea, Hepar sulphuris, Calcium fluoricum, Calcium phosphoricum and Aconite. To close the wounds the calcium pathway is involved also with its inhibitors as Kaliumbichromicum, Mercurius solubilis and Silicea.

Among my patients having had wounds are three interesting cases: 1) one case with 10 bites of an aggressive shepherd dog at the right and left heel; 2) one case with terrible wounds at the shinbone and 3) one case with a lymphangitis after an injury at the lower leg. Here I added lymp glands and Naja tripudians, venom of cobra containing also a choline esterase and cobrotoxin, *Staphylococcus aureus* and *Streptococcus pyrogenes*. The homeopathic remedies had been applied twice a day, sometimes 3 times when it was really worse, at the beginning, then after the wounds had closed and the pain was reduced, the remedies had also been reduced to one application a day, mostly after a week. Totally healing was achieved after 3-4 weeks; important is that the patients had closed wounds and no pain, but they were sensible, they had to pay attention to their state. Homeopathic therapy can be applied without any side effects; it is a therapy with low costs

References

1. Lenger K.,Homeopathic potencies identified by a new magnetic resonance method. Homeopathy-an energetic medicine. Subtle Energies & Energy Medicine, 15, No. 3:225-243, 2006.
2. Lenger K. et.al.,Delayed luminescence of high homeopathic potencies on sugar globuli. Homeopathy; 97, 134-140, 2008
3. Lenger K.,A new biochemical of homeopathic efficacy in patients with chronic diseases.Subtle Energies & Energy Medicine, 19(3), 9-41, 2010.
4. Lenger K. et al. Identification of unknown homeopathic remedies by delayed luminescence. Cell Biochem. Biophys , 68; 321-334, 2014.
5. Lenger K, Lang Günter,Photons detected by magnetic resonance are efficious in homeopathy. A critical review. OA Alternative Medicine, 2(1):4, 2014.
6. Lenger K., Homeopathy—A Regulation Therapy Healing Hypo- or Hyper-functions of Pathological Pathways by Magnetic Photons according to the Resonance Principle. Journal of Life Sciences 11 (2017) doi: 10.17265/1934-7391/2017.11.001

MicroRNA-132, a promising target for wound therapy

X. Li[1] / D. Li[1] / A. Wang[2] / T. Chu[2] / W. Lohcharoenkal[1] / E.K. Herter[1] / L. Eidsmo[1] / E. Sonkoly[1] / A. Pivarcsi[1] / S.-B. Catrina[3] / M. Ståhle[1] / N. Xu Landén[1]

[1]Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden
[2]Department of Dermatology, The Second Hospital of Dalian Medical University, Dalian, China
[3]Department of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital, Stockholm, Sweden

ning.xu@ki.se

Diabetic foot ulcer (DFU) is a major complication of diabetes mellitus and represents an escalating global burden. We have previously shown that microRNA-132 (miR-132) is required for physiologic skin wound healing by facilitating inflammation-proliferation transition in keratinocytes.

Here, we investigated the role of miR-132 in DFU. We found that miR-132 expression was down-regulated in DFU compared to normal human skin wounds. Skin wounds of leptin receptor-deficient (db/db) diabetic mice also displayed reduced miR-132 expression compared to the wild-type mice and local replenishment of miR-132 effectively accelerated wound closure. Moreover, topical application of miR-132 mimics on human *ex vivo* skin wounds significantly enhanced re-epithelialization. The therapeutic effect of miR-132 for DFU was further supported by potentiating migration in human primary dermal fibroblasts (HDFs).

In HDFs, miR-132 expression was induced by TGF-α1. By global transcriptome analysis, we identified RAS signaling pathway as regulated by miR-132 and silencing of RAS p21 protein activator 1 (RASA1) phenocopied miR-132 overexpression in HDFs. Together, our study demonstrated the therapeutic potential of miR-132 in DFU, which warrants further evaluation in controlled clinical trials.

Histological characterization of traumatic neuroma development

K. M. C. Oliveira / L. Pindur / J. H. Barker[1]/ L. Leppik

Frankfurt Initiative for Regenerative Medicine (FIRM) - J.W. Goethe University of Frankfurt, Germany

karlamychellyne@yahoo.com.br

Introduction

Limb amputation is a devastating condition caused by trauma, cancer, and peripheral vascular disease. Pain is the most common complaint of amputee patients, which is mainly caused by sensitized neuroma formation at the site of injury. Neuromas are typically benign neural tumors, which may occur after nerve injury, and can be very painful. While most studies focus on developing pain therapies for already formed neuroma, little is known about the pathophysiology of neuroma formation.

This study was designed to characterize morphologic stages of neuroma development with an eye toward developing better treatment strategies that intervene before neuromas are fully formed.

Results

Right upper limbs of 30 Sprague Dawley rats were amputated and limb stumps were collected at 3, 7, 28, 60 and 90 Days Post Amputation (DPA). Morphology of newly formed nerves and neuromas were assessed via general histology and neurofilament protein antibody (NF) staining. Analysis revealed six well defined morphological characteristics of nerve and neuroma development; 1) normal nerve, 2) axonal sprouts, 3) degenerating fascicles, 4) unorganized bundles of axons, 5) unorganized axon growth into muscles, and 6) unorganized axon growth into fibrotic tissue (neuroma).

At early stages (3 & 7 DPA) after amputation, normal nerves could be identified throughout the limb stump tissue and small areas of axonal sprouts were present in the distal region of stumps near the site of injury.

Signs of degenerating nerves (identified by calculating axonal density in nerve fascicle) were evident from 7 to 90 DPA. From day 28 on, variability of nerve characteristics with signs of unorganized axons into tissues and frank neuroma formation became visible in multiple areas of stump tissue. These pathological features became more evident and evenly distributed on days 60 and 90. At 90 DPA frank neuroma formation was uniformly present in all stump tissue.

Perspectives

By following nerve regrowth and neuroma formation after injury we were able to identify 6 separate histological stages of nerve/neuroma formation. In these preliminary studies we observed axonal regrowth as early as 3 days post amputation and signs of unorganized axonal growth and neuroma formation by 28 DPA. Future research will focus on studying the underlying mechanisms in order to influence neuroma formation in the dynamic early stages of development.

This could lead to development of new and better treatments and/or prevention strategies for patients suffering with painful neuromas.

Selective targeting of Fibroferon to interstitial myofibroblasts attenuates renal fibrosis

F. Poosti[1,5] / R. Bansal[2] / S. Yazdani[2,5] / J. Prakash[2] / L. Beljaars[3] / J. v.d. Born[4] / M. H.d. Borst[4] / H. v. Goor[1] / J. L. Hillebrands[1] / K. Poelstra[3]

[1]Dept. Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, The Netherlands
[2]Dept. Biomaterials Science and Technology, MIRA Institute, University of Twente, The Netherlands
[3]Dept. Pharmacokinetics, Toxicology and Targeting, University of Groningen, The Netherlands
[4]Dept. Internal Medicine, University Medical Center Groningen, University of Groningen, The Netherlands
[5]Dept. Microbiology and Immunology, Rega Institute, KU Leuven, Belgium

fariba.poosti@kuleuven.be

Development of renal fibrosis is the final common pathway of chronic kidney disease (CKD), which ultimately leads to end-stage renal disease (ESRD) demanding renal replacement therapy. No adequate preventive or curative therapy is available that could be clinically used to target renal fibrosis specifically. Thus, there is a great need to develop new therapies to halt fibrogenesis and consequently arrest the progression toward ESRD. Interferon-γ is a pro-inflammatory cytokine with anti-fibrotic properties. Clinical use of interferon-γ is hampered due to inflammation-mediated systemic side effects.

We developed an interferon-γ peptidomimetic (mimγ) lacking the extracellular IFNγ-Receptor recognition domain, and coupled it to the PDGFβR-recognizing peptide BiPPB. Here we investigated the efficacy of mimγ-BiPPB (referred to as “Fibroferon”) targeted to PDGFβR-overexpressing interstitial myofibroblasts to attenuate renal fibrosis without inducing inflammation-mediated side effects in the mouse unilateral ureter obstruction model. Unilateral ureter obstruction (UUO) induced renal fibrosis characterized by significantly increased α-SMA, TGFβ1, fibronectin, and collagens I and III protein and/or mRNA expression. Fibroferon treatment significantly reduced expression of these fibrotic markers.

Compared to full-length IFNγ, anti-fibrotic effects of Fibroferon were more pronounced. UUO-induced lymphangiogenesis was significantly reduced by Fibroferon but not full-length IFNγ. In contrast to full-length IFNγ, Fibroferon did not induce IFNγ-related side-effects as evidenced by preserved low-level brain MHC II expression (similar to vehicle), lowered plasma triglyceride levels, and improved weight gain after unilateral ureter obstruction. In conclusion, compared to full-length IFNγ, the IFNγ-peptidomimetic Fibroferon targeted to PDGFβR-overexpressing myofibroblasts attenuates renal fibrosis in the absence of IFNγ-mediated adverse effects.

MiRNAs regulation and its role as biomarkers in scleroderma

M. Rusek[1,2] / M. Michalska-Jakubus[1] / M. Kowal[1] / J. Bełtowski[2] / D. Krasowska[1]

[1]Medical University of Lublin, Laboratory of Immunology Skin Diseases, Department of Dermatology, Venereology and Pediatric Dermatology, Lublin, Poland
[2]Medical University of Lublin, Department of Pathophysiology, Lublin, Poland

marta.rusek@yahoo.com

MiRNAs are intensively evaluated as promising blood-based biomarkers of diagnostic application[1]. Because miRNAs are relatively high stable in biofluids, such as plasma, serum, and urine and the ability of miRNA expression profiles to accurately classify discrete tissue types and disease states has positioned miRNAs as a promising part of translational medicine[2,3]. MiRNAs are small non-coding RNAs that play an important role in post-transcriptional gene regulation.

Clinical manifestations of systemic sclerosis (SSc) include multiorgan abnormalities affecting skin, joints, muscles, lung, heart, kidney and vasculature, but molecular mechanisms underlying these abnormalities remain obscure contributing to limited efficacy of treatment. SSc is characterized by three pathological processes: cellular/humoral autoimmunity, fibrosis, and vascular changes[4]. Because profile of miRNAs in biofluids have been found to be different in pathological states, we predict that circulating miRNAs would be a great blood-based marker for molecular diagnostics[5].

The study enrolled 10 SSc patients and 6 controls (aged 43-85, 63.10±12.53 yo). We prepared miRNA from serum of these patients and analyzed the miRNA profile by microarrays and qRT-PCR. Hierarchical clustering analysis was performed. Patients and controls sample miRNA levels were compared and differences with a $p<0.05$, false discovery rate (FDR) <0.1 and fold change ±2 were considered statistically significant.

Microarray profiling demonstrated that 7 miRNAs were markedly down- or up-regulated in SSc patients, compared to the control group. The 18-fold change of miR-4484 level might serve as potential biomarker for SSc detection, although it has not been reported in previous studies. Based on bioinformatic studies, the main target genes for miR-4484 are MMP-21, AATF, PTPN14, which play a role in fibrosis, apoptosis and ADCY3 - in hormones signaling pathways respectively. MMPs disrupt the basement membrane, allowing inflammatory cells to be easily recruited to the site of injury. Information regarding the MMPs levels in inflammation process and wound healing enables optimal treatment plans to be developed for individual patients. Additionally, in this study, we found a decrease in miR-584 and miR-7110-5p and an increase in miR-520-g, miR-520-h and miR-4529-3p. These findings demonstrate that gene expression might be regulated by miRNA in SSc patients. Further research is needed to explain the significance of the obtained results.

References

1. R. Duttagupta, R. Jiang, J. Gollub, R.C. Getts, K.W. Jones, Impact of Cellular miRNAs on Circulating miRNA Biomarker Signatures, PLoS One. 6 (2011) 1–14. doi:10.1371/journal.pone.0020769.
2. M.B. Kirschner, N. van Zandwijk, G. Reid, Cell-free microRNAs: potential biomarkers in need of standardized reporting, Front. Genet. 4 (2013). doi:10.3389/fgene.2013.00056.
3. C.C. Pritchard, H.H. Cheng, M. Tewari, MicroRNA profiling: approaches and considerations, Nat. Rev. Genet. 13 (2012) 358–369. doi:10.1038/nrg3198.
4. J.N. Fleming, S.M. Schwartz, The pathology of scleroderma vascular disease, Rheum. Dis. Clin. North Am. 34 (2008) 41–55. doi:10.1016/j.rdc.2008.01.001.
5. E.M. Kroh, R.K. Parkin, P.S. Mitchell, M. Tewari, Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR), Methods. 50 (2010) 298–301. doi:10.1016/j.ymeth.2010.01.032.

Transcription factor complex TRIM33-Smad2/3 is crucial for progenitor cell-mediated liver regeneration in liver cirrhosis

X. Yuan[1] / T. Lin[1] / F. Wandrer[2] / H. Bantel[2] / S. Dooley[1] / H. L. Weng[1]

[1]Department of Medicine II, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany
[2]Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

xiaodong.yuan@medma.uni-heidelberg.de

Introduction

Severe parenchymal loss in the liver induces a massive expansion of liver progenitor cells (LPC), called ductular reaction (DR). However, in this setting, LPC frequently fail to differentiate into functional mature hepatocytes. Incomplete LPC differentiation is one of the main causes of liver failure in patients with liver cirrhosis. Tripartite motif protein (TRIM) 33 is crucial for differentiation of embryonic stem cells through formation of transcription factor complexes with phosphorylated Smad2 and Smad3, the downstream substrates of activated TGF-β signaling.

We hypothesize that LPC exploit a similar TRIM33-Smad2/3 transcription factor complex dependent differentiation mechanism. TGF-β, Smad2 phosphorylation as well as expression of TRIM33 and goosecoid, a master differentiation gene, were examined in liver tissue specimens of 10 patients with decompensated cirrhosis, who received liver transplantation, as well as 10 compensated cirrhosis patients by immunohistochemistry. Macrophage, monocytes, hepatic stellate cells (HSC) and intermediate hepatocyte-like cells (IHLC) were identified by CD68, CD14/16, α-SMA and CK7 staining, respectively.

Results

Immunohistochemical staining revealed that macrophages/monocytes and activated HSC are predominant inflammatory cells surrounding DR in both compensated and decompensated cirrhotic patients. Macrophages, but not HSC, produced TGF-β. Robust expression of goosecoid was present in hepatocyte buds (comprising DR, IHLC and hepatocytes) of 10 patients with compensated cirrhosis, indicating occurrence of differentiation of IHLC and hepatocytes. These cells showed both p-Smad2 and TRIM33 immunopositive staining in nuclei. In contrast, no TRIM33 and goosecoid expression was found in liver cells of patients with decompensated cirrhosis, although 5 of these showed nuclear p-Smad2 positive staining in LPC.

Perspectives

This preliminary clinical study suggests that lack of TRIM33-Smad2/3 complexes may result in a disturbed differentiation of LPC to mature hepatocytes. Further details of this mechanism in liver regeneration are subject matter of current investigations.

Notes

33rd Ernst Klenk Symposium in Molecular Medicine
Tissue Regeneration, wound healing and fibrosis - Translating basic concepts into regenerative therapy
Oct. 15 – 17, 2017

Overview of the guest speakers

Scientific Coordinator and Key Note Speaker of the Klenk-Lecture 2017

Werner, Sabine - Prof. Dr.
Dept. of Biology, Institute for Molecular Health Sciences, ETH Zurich
Zurich, CH
e-mail: sabine.werner@biol.ethz.ch
http://www.mhs.biol.ethz.ch/research/werner.html/

Guest Speakers

Augustin, Hellmut - PhD, Prof. Dr. med. vet.
Vascular Oncology and Metastasis, German Cancer Research Center (DKFZ)
Heidelberg, DE
e-mail: h.augustin@dkfz-heidelberg.de
http://www.dkfz.de/en/angiogenese/index.php

Guest speaker - Session I
Exploring molecular concepts of cell renewal and stem cell function in tissue maintenance and repair

Ávila, Matías A – PhD, Prof.
Hepatology Programme, Center for Applied Medical Research (CIMA), University of Navarra
Pamplona, ES
e-mail: maavila@unav.es
http://cima.unav.edu/en/investigacion/programas-verticales/hepatologia

Guest speaker - Session III
New strategies of targeting the immune response in tissue remodeling

Bakkers, Jeroen - PhD, Prof.
Dept. of Cardiac Development and Genetics, Hubrecht Institute, Univ. Med. Centre Utrecht
Utrecht, NL
e-mail: j.bakkers@hubrecht.eu
https://www.hubrecht.eu/onderzoekers/bakkers-group/#extraBlock

Guest speaker - Session IV
Challenges and perspectives for translating scientific discoveries in regenerative medicine

Corn, Jacob E - PhD, Prof.
Department of Molecular and Cell Biology, University of California, Berkeley
Berkeley, US
e-mail: jcorn@berkeley.edu
https://cornlab.com/

Guest speaker - Session IV
Challenges and perspectives for translating scientific discoveries in regenerative medicine

Dotto, Gian-Paolo - MD PhD, Prof. Dr.
Dept. of Biochemistry, Univ. of Lausanne, Lausanne, CH
and
Cutaneous Biology Research Center, Massachusetts General Hospital, Charlestown, US
e-mail: paolo.dotto@unil.ch
http://www.unil.ch/ib/home/menuinst/research/dotto--gian-paolo.html

Guest speaker - Session II
Mechanisms of cellular communication in tissue regeneration, repair and fibrosis

Heikenwälder, Mathias - Prof. Dr. rer. nat.
Division of Chronic Inflammation and Cancer, German Cancer Research Center (DKFZ)
Heidelberg, DE
e-mail: m.heikenwaelder@dkfz.de
https://www.dkfz.de/de/chronische-entzuendung-und-krebs/team/heikenwaelder.html

Guest speaker - Session III
New strategies of targeting the immune response in tissue remodeling

Kirsner, Robert S - MD PhD, Prof.
Dept. of Dermatology and Cutaneous Surgery, University of Miami, Miller School of Medicine,
Miami, US
e-mail: rkirsner@miami.edu
http://dermatology.med.miami.edu/kirsner-lab/

Guest speaker - Session IV
Challenges and perspectives for translating scientific discoveries in regenerative medicine

Kubes, Paul - PhD, Prof.
Snyder Institute for Chronic Diseases, University of Calgary
Calgary, CA
e-mail: pkubes@ucalgary.ca
https://www.ucalgary.ca/paulkubeslab/

Guest speaker - Session III
New strategies of targeting the immune response in tissue remodeling

Longaker, Michael T - MD MBA FCA, Prof.
Hagey Laboratory for Pediatric Regenerative Medicine
Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine
Stanford, US
e-mail: longaker@stanford.edu
https://med.stanford.edu/stemcell/about/Laboratories/longaker.html

Guest speaker - Session II
Mechanisms of cellular communication in tissue regeneration, repair and fibrosis

Lutolf, Matthias P - PhD, Prof.
School of Basic Science, École Polytechnique Fédérale de Lausanne
Lausanne, CH
e-mail: matthias.lutolf@epfl.ch
http://lscb.epfl.ch/

Guest speaker - Session I
Exploring molecular concepts of cell renewal and stem cell function in tissue maintenance and repair

Macara, Ian G - PhD, Prof.
Dept. of Cell and Developmental Biology, Vanderbilt University School of Medicine
Nashville, US
e-mail: ian.g.macara@Vanderbilt.Edu/
https://medschool.vanderbilt.edu/macara-lab

Guest speaker - Session I
Exploring molecular concepts of cell renewal and stem cell function in tissue maintenance and repair

Martin, Paul - PhD, Prof. Dr.
Dept. of Physiology & Biochemistry, School of Medical Sciences, University of Bristol
Bristol, UK
e-mail: paul.martin@bristol.ac.uk
http://www.bristol.ac.uk/biochemistry/research/martin-group/

Guest speaker - Session I
Exploring molecular concepts of cell renewal and stem cell function in tissue maintenance and repair

Muñoz-Cánoves, Pura - PhD, Prof.
Department of Experimental and Health Science, ICREA and Pompeu Fabra University
Barcelona, ES
e-mail: pura.munoz@upf.edu
https://www.upf.edu/web/cellbiology

Guest speaker - Session II
Mechanisms of cellular communication in tissue regeneration, repair and fibrosis

Newmark, Phillip A - PhD, Prof.
Howard Hughes Medical Institute and Morgridge Institute for Research, University of Wisconsin
Madison, US
e-mail: pnewmark@morgridge.org
https://morgridge.org/newmark-lab/

Guest speaker - Session I
Exploring molecular concepts of cell renewal and stem cell function in tissue maintenance and repair

Ornitz, David M - MD PhD, Prof.
Dept. of Developmental Biology, Washington University School of Medicine
St. Louis, US
e-mail: dornitz@wustl.edu
http://ornitzlab.wustl.edu/

Guest speaker - Session II
Mechanisms of cellular communication in tissue regeneration, repair and fibrosis

Paro, Renato - PhD, Prof.
Dept. of Biosystems Science and Engineering, ETH Zurich / Faculty of Science, Univ. of Basel
Basel, CH
e-mail: renato.paro@bsse.ethz.ch
https://www.bsse.ethz.ch/egg

Guest speaker - Session I
Exploring molecular concepts of cell renewal and stem cell function in tissue maintenance and repair

Pearce, Edward J - PhD, Prof.
Dept. of Immunometabolism, Max Planck Institute of Immunobiology and Epigenetics and Faculty of Biology, University of Freiburg
Freiburg, DE
e-mail: pearceed@ie-freiburg.mpg.de
http://www.ie-freiburg.mpg.de/4631292/Labor_Ed_Pearce

Guest speaker - Session III
New strategies of targeting the immune response in tissue remodeling

Pollard, Jeffrey W - PhD, FMedSci, FRSE, Prof.
MRC and University of Edinburgh Centre for Reproductive Health and Queen's Medical Research Institute
Edinburgh, UK
e-mail: jeff.pollard@ed.ac.uk
http://www.ed.ac.uk/centre-reproductive-health/professor-jeffrey-pollard

Guest speaker - Session IV
Challenges and perspectives for translating scientific discoveries in regenerative medicine

Roop, Dennis R - PhD, Prof.
Dept. of Dermatology and Charles C. Gates Center for Regenerative, Medicine School of Medicine, University of Colorado
Aurora, US
e-mail: Dennis.Roop@ucdenver.edu
http://www.ucdenver.edu/academics/colleges/medicalschool/centers/StemCell/Faculty/Pages/Denis-Roop.aspx

Guest speaker - Session IV
Challenges and perspectives for translating scientific discoveries in regenerative medicine

Rosenthal, Nadia A - PhD, FMedSci, Prof.
The Jackson Laboratory
Bar Harbor, US
e-mail: nadia.rosenthal@jax.org
https://www.jax.org/research-and-faculty/research-labs/the-rosenthal-lab

Guest speaker - Session III
New strategies of targeting the immune response in tissue remodeling

Sackstein, Robert - MD PhD, Prof.
Dept. of Dermatology and Harvard Skin Disease Research Center,
Brigham and Women's Hospital and Harvard Medical School
Boston, US
e-mail: rsackstein@rics.bwh.harvard.edu
http://www.dfhcc.harvard.edu/insider/member-detail/member/robert-sackstein-md-Ph.D/

Guest speaker - Session IV
Challenges and perspectives for translating scientific discoveries in regenerative medicine

Sordella, Raffaella - PhD, Prof.
Cold Spring Harbor Laboratory
Cold Spring Harbor, US
e-mail: sordella@cshl.edu
https://www.cshl.edu/Faculty/Raffaella-Sordella.html

Guest speaker - Session II
Mechanisms of cellular communication in tissue regeneration, repair and fibrosis

Spatz, Joachim P - Prof. Dr. rer. nat.
Dept. of Cellular Biophysics, Max-Planck-Institute for Medical Research and
Dept. of Biophysical Chemistry, University of Heidelberg
Heidelberg, DE
e-mail: joachim.spatz@is.mpg.de
https://www.is.mpg.de/spatz/

Guest Speaker - Session II
Mechanisms of cellular communication in tissue regeneration, repair and fibrosis

Wynn, Thomas A - PhD, Prof.
Immunopathogenesis Section, Laboratory of Parasitic Diseases
National Institute of Allergy and Infectious Diseases, National Institute of Health
Bethesda, US
e-mail: tom.wynn@nih.gov
https://www.niaid.nih.gov/research/thomas-wynn-Ph.D/

Guest speaker - Session III
New strategies of targeting the immune response in tissue remodeling

Sponsors

MORE POWER TO YOUR PROTEIN RESEARCH

Protein purification and quantification from Bio-Rad

NGC™ Chromatography Systems

Reinvent your approach to protein purification with a system that simplifies method development and adapts and expands to your needs.

ChemiDoc™ MP Imaging System

Experience unmatched fluorescence and chemiluminescence performance with powerful multiplexing capabilities and a wide range of fluorophore options, including NIR dyes.

To find out more, visit the Bio-Rad stand at Ernst Klenk Symposium 2017

BIO-RAD

GUIDE-IT™ PRODUCTS FOR CRISPR/CAS9

Concerned about CRISPR off-target effects?

that's GOOD science!®

Achieve footprint-free gene editing via efficient delivery of Cas9/sgRNA ribonucleoprotein (RNP) complexes

Persistent and elevated expression of Cas9 in target cells may lead to off-target effects. In contrast, when delivering Cas9/sgRNA RNP complexes, the short residence time of the Cas9 protein will greatly increase the specificity of gene editing.

Electroporation of our **Guide-it™ Recombinant Cas9** is a simple method to achieve footprint-free editing. Alternatively, you may consider delivering RNP complexes via cell-derived nanovesicles, called gesicles. Our **Guide-it™ CRISPR/Cas9 Gesicle Production System** is designed to generate gesicles, loaded with Cas9/sgRNA RNP complexes against the target of your choice.

- Efficient delivery of active Cas9 protein and target-specific sgRNA to a broad range of cell types
- Delivery of Cas9 protein eliminates genomic integration and reduces off-target effects
- Tight control over dose and timing of delivery and editing

Learn more at www.takarabio.com/Guide-it

寶 TaKaRa

Clontech TaKaRa cellartis

www.takarabio.com

LA ROCHE-POSAY
LABORATOIRE DERMATOLOGIQUE

FÜR ÄRZTE AUS KLINIK UND PRAXIS IN WEITERBILDUNG ZUM FACHARZT DERMATOLOGIE

Seit vielen Jahren fördert La Roche-Posay
spezifische Fortbildungsangebote für junge Ärzte:

Spannende Vorträge, praktische Anwendungen
sowie ein intensiver Informationsaustausch warten auf Sie.

Nehmen Sie teil an dieser erstklassigen Verbindung aus Theorie und Praxis.



INTERESSE GEWECKT? SCHREIBEN SIE MIR:

Dr. med. Irene Rosengarten
Med. - Wiss. Leitung

E-Mail: Irene.ROSENGARTEN@loreal.com

www.assistentenforum.de

Organization

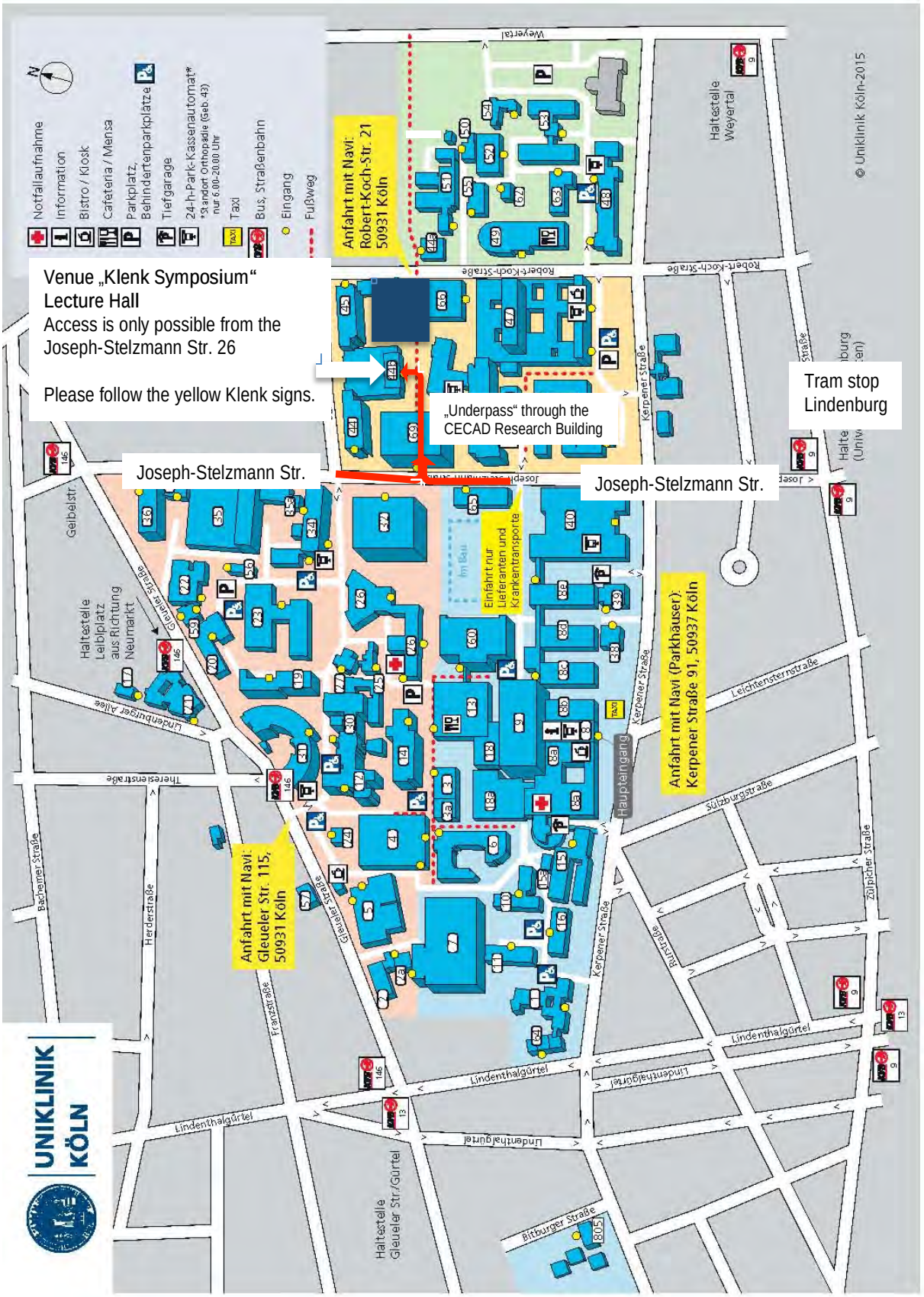
Dr. rer. nat. Debora Grosskopf-Kroiher
Scientific Coordinator / Managing Director

CMMC Office Team
Konstantin Belostotski • Tanja Bertenburg • Kathleen Geiser • Dr. Claudia Herr • Bettina Kranz
Angelika Winterscheid • Tina Hafermann • Halina Weinert • Georgiana Klug-Micu

We gratefully acknowledge the organizational support during the Klenk Symposium 2017 by members of the CMMC´s Research Groups and Core Facilities, the Institute for Vegetative Physiology, the Center for Biochemistry and the "Regionales Rechenzentrum (RRZK)" of the University of Cologne.

Photography
© davis, stock.adobe.com/de - front cover
Lars Rubert (University of Cologne) - front cover, inside

Site map - Campus
Medical Faculty, Univ. Cologne / Uniklinik Köln



33rd Ernst Klenk Symposium in Molecular Medicine

Tissue regeneration, wound healing and fibrosis: Translating basic concepts into regenerative therapy

Oct. 15 - 17, 2017

Venue

Lecture Hall of the Medical Faculty
Access is only possible from Joseph-Stelzmann-Str. 26, 50931 Cologne.
Please follow the yellow Klenk signs.

Program Overview

Sunday - Oct. 15, 2017	
12.50 p.m.	Start of the Klenk Symposium - Welcome Address
Session I	Exploring molecular concepts of cell renewal and stem cell function in tissue maintenance and repair
1.00 - 2.30 p.m.	Part I - Paul Martin - Renato Paro - Phillip A Newmark
2.30 - 3.00 p.m.	Coffee Break
3.00 - 4.30 p.m.	Part II - Ian G Macara - Matthias P Lutolf - Hellmut Augustin
4.30 - 5.00 p.m.	Coffee Break
5.00 - 8.30 p.m.	Poster Session and Welcome Get-Together *with free finger food and drinks for all participants*

Monday - Oct. 16, 2017	
Session II	Mechanisms of cellular communication in tissue regeneration, repair and fibrosis
9.00 - 10.30 a.m.	Part I - Michael T Longaker - Joachim P Spatz - Pura Munoz-Canoves
10.30 - 11.00 a.m.	Coffee Break
11.00 - 12.30 a.m.	Part II - David M Ornitz - Raffaella Sordella - Gian-Paolo Dotto
12.30 - 2.00 p.m.	Lunch Break – free soup and sandwiches for all participants
Session III	New strategies of targeting the immune response in tissue remodeling
2.00 - 3.30 p.m.	Part I - Thomas A Wynn - Edward J Pearce - Paul Kubes
3.30 - 3.50 p.m.	Coffee Break
3.50 - 4.00 p.m.	Announcement of the Poster Awards - Sabine Eming and Catherin Niemann
4.00 - 5.30 p.m.	Part II - Matias A Avila - Nadia A Rosenthal - Mathias Heikenwälder
5.30 - 6.00 p.m.	Coffee Break
6.00 - 7.00 p.m.	Ernst Klenk Lecture - Sabine Werner

Tuesday - Oct. 17, 2017	
Session IV	Challenges and perspectives for translating scientific discoveries in regenerative medicine
9.00 - 10.30 a.m.	Part I - Robert S Kirsner - Dennis R Roop - Robert Sackstein
10.30 - 11.00 a.m.	Coffee Break
11.00 - 12.30 a.m.	Part II - Jeffrey W Pollard - Jeroen Bakkers - Jacob E Corn
12.30 - 1.00 p.m.	Short presentation by the three poster awardees (3 x 10 min. short talk)
1.00 - 1.15 p.m.	Summary, open questions, concluding remarks