Thieme

IV. MuSkITYR Symposium

Datum/Ort:
09.–10. November 2022, Frankfurt

Kongresspräsidenten:
Kai Böker; Felix Schmidt; Marietta Herrmann

1 Frakturheilung in Kombination mit schwerem Blutverlust – Welchen Effekt hat das Alter?

Autoren Bundkirchen K[1], Ye W[1], Xu B[2], Relja B[2], Neunaber C[1]
Institute **1** Unfallchirurgie, Medizinische Hochschule Hannover, Hannover, Deutschland; **2** Radiologie, Otto-von-Guericke Universität Magdeburg, Magdeburg, Deutschland
DOI 10.1055/s-0042-1757521

Einleitung Bei der Heilung von Frakturen (Fx) assoziiert mit starken Blutungen treten häufig Komplikationen auf [1]. Dies ist vor allem bei älteren Patienten zu beobachten [2–5]. Da diese Begleitumstände den klinischen Behandlungsverlauf stark beeinflussen, wurde in dieser Studie der Einfluss vom Alter auf die Knochenregeneration nach Femurfraktur und schwerem Blutverlust untersucht.

Material und Methodik Die Gruppe Fx wurde einer Osteotomie nach Anlegen eines externen Fixateurs unterzogen. Die Gruppe THFx erhielt zusätzlich eine Blutdruck-kontrollierte Trauma-Hämorrhagie (TH). Der Gruppe Sham wurden lediglich Katheter, sowie Fixateur implantiert. Es wurden µCT-Scans, histologische, sowie biomechanische Untersuchungen 3 Wochen nach Trauma durchgeführt. Die Analyse der nicht-normalverteilten Daten erfolgte mittels Mann-Whitney-U- bzw. Kruskal-Wallis-Test und die statistische Signifikanz wurde auf $p < 0.05$ festgesetzt.

Ergebnisse Die Histologie ergab weniger mineralisierten Knochen in alten Tieren der Gruppen Fx ($p = 0{,}003$) und THFx ($p = 0{,}009$) verglichen mit den Jungen. Ein Blutverlust führte weiterhin in jungen ($p = 0{,}004$) und alten Tieren ($p = 0{,}032$) zu mehr Knorpel im Vergleich zu Fx. In alten Tieren waren im Gegensatz zu den jungen Mäusen mit Fx weniger Osteoklasten vorhanden ($p = 0{,}009$), sodass der schwere Blutverlust lediglich in jungen Tieren zu einer Reduzierung der Osteoklastenanzahl führte ($p = 0{,}004$). Die µCT-Scans zeigten in alten THFx-Tieren einen reduzierten Kallusanteil ($p = 0{,}030$), sowie eine geringe Trabekelanzahl ($p = 0{,}041$) im Vergleich zu Fx. Ein verringertes Elastizitätslimit der alten THFx Mäuse im Gegensatz zu den alten Fx konnte herausgestellt werden ($p = 0{,}022$).

Zusammenfassung Ein schwerer Blutverlust hat einen stärkeren negativen Effekt im Hinblick auf die Heilung, die Morphometrie und die biomechanischen Eigenschaften frakturierter Femora in alten Mäusen verglichen mit jungen, adulten Tieren.

Interessenskonflikt Es liegt kein Interessenkonflikt vor.

Literatur

[1] Marx et al. Die Intensivmedizin: Springer 2015
[2] Clark D et al. Curr Osteoporos Rep. 2017; 15 (6): 601–8
[3] Meinberg EG et al. Injury 2019; 50: (Suppl 1): S62–5
[4] Gorter EA et al. Bone reports 2021; 15: 101117
[5] Borgström F et al. Arch Osteoporos 2020; 15 (1): 59

2 The role of Wnt and Rsk2 pathways in controlling osteosarcoma development in a *c-Fos* transgenic mouse model.

Authors Carreau A[1], Baldauf C[1], Neven M[1], David JP[1], Schinke T[1]
Institute **1** Institute of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf
DOI 10.1055/s-0042-1757522

Introduction *c-Fos* transgenic mice that overexpress the transgene under the control of the H2kb promoter have been described to develop spontaneous primary bone tumors: Osteosarcomas (OS) due to the transformation of cells of the osteoblastic lineage [1]. The ribosomal S6 kinase 2 (Rsk2), responsible of an activating phosphorylation of Fos, is known to be essential for the growth of cFos-induced osteosarcoma in mouse [2]. However the mechanism by which Rsk2 controls tumor development and whether Rsk inhibitors could be used in treatment is unknown. In addition, we analyzed if the bone anabolic Wnt pathway affects osteosarcoma development.

Material and Methods We created cell lines of osteosarcomas of cFosTg mice crossed withRsk2 deficient mice (FosTg;Rsk2$^{-/-}$). The results were compared to Fostg mice crossed with knock out mice of LRP5,a co-receptor for Wnt signaling (FosTg;LRP5$^{-/-}$) or with LRP5 knock in mice (fosTg;LRP5$^{A213V/+)}$, showing low or high bone mass, respectively.

Results Our results showed that FosTg;Rsk2$^{-/-}$ cells have an impaired growth avantage compared to Fostg cells. Analysis of their DNA content and microscopy observation revealed aberrant nuclei numbers leading to a "mitotic catastrophe". FosTg;LRP5$^{-/-}$ mice show a reduction of the overall number of tumors of the skeleton and the tumor volume was drastically reduced similar to the observation made in vivo by deletion of Rsk2. In the high bone mass model, we observed, most importantly, an increase in the tumor volume. Cell lines created of these 3 mouse models, however, didn't show any growth or mineralization impairment.

Conclusion Our analysis show that Wnt and Rsk2 impair osteosarcoma development in the c-fos transgenic mice by different mechanisms.

Conflict of Interest No conflict.

References

[1] Grigoriadis AE et al. J Cell Biol. 1993; 122 (3): 685–701
[2] Jean-Pierre D et al. J Clin Invest. 2005; 115 (3): 664–672

3 Analysis of communication between muscle and nerve cells

Authors Conradi-Meyer C[1], Böker KO[1], Lehmann W[1], Schilling AF[1]
Institute **1** Universitätsmedizin Göttingen, Klinik für Unfallchirurgie, Orthopädie und plast. Chirurgie, Göttingen, Deutschland
DOI 10.1055/s-0042-1757523

Introduction Peripheral nerves are often affected by trauma. Regeneration is mostly slow and incomplete. The prerequisite for an efficient regeneration is Wallerian degeneration, as well as support by growth factors [1]. The extent to which muscle training influences regeneration, e. g. by increased secretion of growth factors, is not yet sufficiently understood. Therefore, this project investigates the influence of muscle contraction on neurite growth in an *in vitro* model.

Osteologie 2022; 31: e2–e8

Material and Methods Muscle cells (C2C12) were differentiated into myotubes and stimulated with electric pulses, to trigger myotube contraction. Afterwards, the myotube stimulation medium was collected. Furthermore, differentiation was induced in neuroblastoma cells (SH-SY5Y) with the aid of retinoic acid. These SH-SY5Y cells were treated with medium of stimulated and unstimulated myotubes to observe effects on neurite growth. After several days of cultivation in myotube medium, microscopy images were taken. The images were evaluated using an ImageJ plugin that automatically measures neurite lengths.

Results We established a system to stimulate myotubes in cell culture in our labor. Contraction can be observed under the microscope while the myotubes are stimulated in a 6-Wellplate with electrodes. To confirm the system does not damage the cells, we ran a lactate dehydrogenase (LDH) Assay. No significantly higher LDH concentration was detected in the cell culture medium of stimulated myotubes, which proves the system is not cell toxic. Furthermore, the length of neurites can be measured automatically with an ImageJ Plugin now, instead of manually measuring it.

Conclusion Systems for electrical pulse stimulation and neurite length measurement are established. The next step is to run the experiment described in methods, to finally observe effects of myotube medium (paracrine effects) on neurite growth.

Conflict of Interest No conflict

References

[1] Burnett MG et al. Neurosurg Focus 2004; 16: 1–7

4 Einfluss einer CD9-Überexpression auf die Aktivität von Osteoklasten

Autoren Freiin von Saß LK[1], Böker KO[1], Schilling AF[1], Lehmann W[1]

Institut 1 Klinik für Unfallchirurgie, Orthopädie und plastische Chirurgie, Universitätsmedizin Göttingen

DOI 10.1055/s-0042-1757524

Einleitung Erkrankungen mit verminderter Knochendichte, wie Osteoporose, liegt eine übermäßige Aktivität der knochenabbauenden Osteoklasten zugrunde [1], welche durch Fusion von monozytären Vorläuferzellen entstehen [2]. Bei Membranfusionen spielt das membranständige Tetraspanin CD9 eine entscheidende Rolle [3]. Während der in vitro Osteoklastogenese wurde beobachtet, dass sich der Gehalt an CD9 verändert [4]. Unklar ist, ob eine dauerhafte CD9-Überexpression einen Einfluss auf die Morphologie und Aktivität von reifen Osteoklasten hat.

Material und Methodik Zur Überprüfung der Hypothesen wurde zunächst CD9, GFP und eine Blasticidinresistenz mittels eines lentiviralen Vektors in das Genom einer humanen, monozytären Zelllinie (THP1) transduziert. Das führte zu einer kontinuierlichen Überexpression des membranständigen Tetraspanins CD9. Durch ein blasticidinhaltiges Medium wurden die Zellen weiter selektiert. Der Gehalt an CD9 wurde mittels qPCR und Western Blot dargestellt. Anschließend folgte die Differenzierung der Monozyten mithilfe von PMA, RANKL und M-CSF zu reifen Osteoklasten.

Ergebnisse Die Transduktion von CD9 zeigte in Western Blot und qPCR eine 5-fache CD9-Expression, die mithilfe antibiotischer Selektion verstärkt wurde. Ein morphologischer Unterschied der werdenden Osteoklasten im Vergleich zum THP1-Wildtyp ist bereits in den frühen Differenzierungsstadien erkennbar.

Zusammenfassung CD9 spielt während der Osteoklastogenese eine entscheidende Rolle. Durch einen lentiviralen Vektor und weitere Selektion konnten wir eine stabile CD9-Überexpression in Monozyten erreichen und diese zu Osteoklasten differenzieren. Unterschiede bezüglich der Aktivität der Osteoklasten sind Teil der laufenden Untersuchungen. Hat CD9 einen Einfluss auf die Osteoklastenaktivität, wäre dies ein potenzielles Ziel für eine immunmodulierende Therapie der Osteoporose, um die Aktivität von Osteoklasten herabzusetzen.

Interessenskonflikt Kein.

Literatur

[1] Lane JM et al. Clin Orthop Relat Res. 2000; 372: 139–50
[2] Boyle WJ et al. Nature 2003; 423 (6937): 337–42
[3] Miyado K et al. Science 2000; 287 (5451): 321–4
[4] Ishii M et al. J Bone Miner Res 2006; 21 (6): 965–76

5 Influence of a AAV-mediated Foxo3 knockdown on myogenic differentiation

Authors Gellhaus B[1], Böker KO[1], Rodenwaldt E[1], Lehmann W[1], Schilling AF[1], Saul D[1, 2, 3]

Institutes 1 Clinic for Trauma Surgery, Orthopaedic Surgery and Plastic Surgery, Universitätsmedizin Göttingen, Göttingen 37075, Germany; 2 Division of Endocrinology, Mayo Clinic, Rochester, MN, 55905, USA; 3 Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN, 55905, USA

DOI 10.1055/s-0042-1757525

Introduction Sarcopenia is defined as a reduction of muscle strength due to aging that is of tremendous importance for an aging population [1]. As an important driver of the ubiquitin-proteasomal system, the E3 ubiquitin ligase Fbxo32, is controlled by FOXO3, leading to proteasomal protein degradation [2]. The clinical relevance of sarcopenia-induced falls is pervasive: Impaired gait safety leads to increased risk of falling, leading to fractures [3]. Therefore, it is limiting the quality of life.

Material and Methods Murine C2C12 myoblasts were transduced with AAVs delivering a siRNA against the *Foxo3* mRNA and a functionless siRNA as control. Knockdown efficiency was increased by establishing FACS sorted single cell lines. They were screened for *Foxo3* knockdown by qPCR. The FOXO3 knockdown was confirmed on protein level by western blot. Transduced cells were seeded and differentiated into myotubes under low serum conditions. Differentiated myotubes were stained for myosin heavy chain by immunocytochemistry. Morphological analysis was performed using an ImageJ macro to characterize the myotube size and nuclei fusion index.

Results The transduction of a siRNA against the *Foxo3* mRNA led to a *Foxo3* overexpression ($p < 0.0001$) on transcriptional level from day 5 during differentiation. But on protein level we reached a stable and significant long-term FOXO3 knockdown ($p < 0.05$) from day 3 on. Further, the *Foxo3* knockdown myotubes showed a smaller phenotype ($p < 0.05$) and a lower nuclei fusion index ($p < 0.0001$).

Conclusion FOXO3 seems to play an important role in myogenic differentiation. Mechanism and explanations are part of our recent ongoing study.

Conflict of Interest No conflicts of interest to declare

References

[1] Cruz-Jentoft et al. Age Ageing 2019; 48 (1): 16–31
[2] Sandri et al. Cell 2004; 117 (3): 399–412
[3] Bischoff-Ferrari et al. Osteoporos Int 2015; 26 (12): 2793–802

6 Mapping the femoral bone marrow mononuclear cell microenvironment

Authors Häusner S[1, 2], Horas K[3], Rudert M[3], Herrmann M[1, 2]

Institutes 1 IZKF Group Tissue Regeneration in Musculoskeletal Diseases, Interdisciplinary Centre for Clinical Research (IZKF), University Hospital of Wuerzburg, Germany; 2 Bernhard-Heine-Center for Locomotion Research, University of Wuerzburg, Germany; 3 Department of Orthopaedic Surgery, König-Ludwig-Haus, University of Wuerzburg, Germany

DOI 10.1055/s-0042-1757526

Introduction Autologous bone grafting remains the gold standard, especially in demanding long segmental bone defect reconstruction and non-union cases [1]. Composed of a heterogenous cell population, growth factors and various extracellular matrix proteins, autologous bone marrow grafts make up for a nearly perfect regeneration triad. While used as definitive treatment in routine clinical application, its native composition, cellularity and mode of action is still elusive [2]. In this approach especially the fraction of femoral bone marrow mononuclear cells (BMNCs) was investigated in flow cytometry by staining with different extracellular markers in order to characterize specific cell subpopula-

tions. Extensive knowledge about BMNCs cellularity will act as basis to facilitate the translational process from pre-clinically developed protocols towards new therapeutic strategies for bone regeneration.

Material and Methods Human bone marrow samples (n = 5) were obtained during hip arthroplasty by metaphyseal femoral punching with patient's full written consent and ethical approval. Samples were initially processed after surgery and BMNCs isolated by density gradient centrifugation. Subsequently, cells were processed for multi-parametric flow cytometry analyses. Three main marker panels (P) were predefined for cells of myeloid (P1 e.g. CD80, CD163, CD11b) lymphoid (P2 e.g. CD3, CD4, CD8, CD25) and endothelial and skeletal precursor cell (P3 e.g. CD31, CD133, CD45, CD271) lineage.

Results Empirical analyses reveals distinct populations of ubiquitous cell types present in all patient samples: e.g. P1: CD11b (80 %), CD14 (14,9 %), P2: CD3 (9,72 %), P3: CD45 + (39,9 %) but also cells of specific tissue niches e.g. tissue-resident macrophages CD163 + /CD169 + (0,362 %) and mesenchymal stromal cells (MSCs) CD45-/CD271 + (0,11 %).

Conclusion The preliminary data sets show comparable levels of cells throughout all main and subpopulations of all femoral punch samples, despite patient variability. The identified cell types seem to be feasible drug targets for direct and indirect applications to improve BMNCs osteogenic and angiogenic properties in the autograft.

Conflict of Interest The authors have no Conflict of Interest to declare.

References

[1] Schmidt AH. Injury 2021; 52: (Suppl 2): S18–S22
[2] Bara JJ et al. Cytotherapy 2015; 17 (4): 458–72

7 Mesenchymal stromal cell senescence in multiple myeloma: what came first?

Authors Ilic J[1, 2], Schlierf B[1, 2], Rudert M[2, 3], Ebert R[2], Trivanovic D[1, 2], Herrmann M[1, 2]

Institutes **1** IZKF Group Tissue Regeneration in Musculoskeletal Diseases, University Hospital Wuerzburg, Germany; **2** Bernhard-Heine-Center for Locomotion Research, University of Wuerzburg, Germany; **3** Department of Orthopaedic Surgery, König-Ludwig-Haus, University of Wuerzburg, Germany

DOI 10.1055/s-0042-1757527

Introduction Mesenchymal stromal cells (MSCs) strongly regulate the outgrowth of malignant plasma cells – multiple myeloma (MM) cells. MM, as well as aging, is linked to MSCs' senescence, influencing their functionality and role in cancer progression.

Material and Methods MSCs are isolated from bone marrow of patients undergoing hip arthroplasty. Their viability and proliferation are examined upon treatments with senolytics quercetin and dasatinib, cytostatic bortezomib and co-cultures with MM cells upon 72 h, via MTT assay and flow cytometry. Metabolic status and senescence specific markers are analyzed with cellular assays and histochemical staining.

Results Quercetin and dasatinib decreased the metabolic activity of MSCs significantly, without an effect on their cell cycle. Addition of bortezomib did not influence the senolytic-reduced MSCs growth upon 72 h of treatment. However, when co-cultured with MM cells and treated with bortezomib, MSCs show a decrease in the expression of Ki67, not detectable when only bortezomib is applied. In addition, in co-culture with senescent MSCs, presence of MM cells and bortezomib significantly increased the percentage of β-galactosidase activity in MSCs.

Conclusion Our results imply an inhibitory effect MM cells bring to MSC proliferation, particularly when treated with bortezomib, indicating their role in causing a pro-inflammatory senescent phenotype of MSCs. Here, we revealed that the initial replicative state of MSCs is patient-specific, highly variable and dictates their response, which our further research aims to unveil in more detail.

Conflict of Interest The authors confirm no Conflict of Interest.

8 Entwicklung eines auf maschinellem Lernen basierenden Vorhersagemodells zur Abschätzung des Timed up and Go Tests bei orthogeriatrischen Patienten

Autoren Kraus M[1,*], Stumpf UC[1], Keppler AM[1], Neuerburg C[1], Böcker W[1], Wackerhage H[2], Baumbach SF[1], Saller MM[1]

Institute **1** Musculoskeletal University Center Munich, Department of Orthopaedics and Trauma Surgery, University Hospital, Ludwig-Maximilians-University (LMU), Munich-, -Germany; **2** Faculty of Sport and Health Sciences, Technical University of Munich, Munich, Germany

DOI 10.1055/s-0042-1757528

Einleitung Die Messung der körperlichen Gebrechlichkeit bei älteren Patienten mit orthopädischen Problemen ist derzeit entweder subjektiv, unzuverlässig, zeitaufwendig oder nur bei unverletzten Patienten möglich. Die beiden Ziele der Studie waren daher die Entwicklung eines objektiven, multifaktorielle machine-learning Modells das nicht auf Mobilitätsdaten angewiesen ist, und die Validierung des Modells durch Messung der Validität für die Ergebnisse orthogeriatrischer Patienten während des Timed-up-and-Go-Test (TUG).

Material und Methodik Es wurden 6 verschiedene Algorithmen zur Variablenselektion aus 67 multifaktorielle, Parameter verwendet, um 4 verschiedene maschinelle Lernalgorithmen zu trainieren. Hierbei konnten Daten von 102 Probanden in die Auswertungen eingeschlossen werden. Ein lineares Modell, eine Support-Vektor-Maschine, ein Random-Forest-Algorithmus und ein Extrem-Gradient-Boost-Algorithmus wurden verwendet, um die für den Timed-up-and-Go-Test benötigte Zeit vorherzusagen, ohne dabei auf Mobilitätsdaten zurückzugreifen.

Ergebnisse Bei unserem aktuellen Datensatz liefert der Random-Forest-Algorithmus die besten Ergebnisse bei der Schätzung der TUG-Zeit, mit einem mittleren absoluten Fehler von 2,7s für den besten Algorithmus und 7,8s für den schlechtesten Algorithmus. Die für die Variablenauswahl verwendete Methodik scheint nur einen geringen Einfluss auf die endgültige Performance zu haben. Alle verwendeten Algorithmen überschätzen die Zeit bei schnellen Patienten und unterschätzen die Dauer bei langsamen Patienten.

Zusammenfassung Es ist möglich, die TUG-Zeit mit einem maschinellen Lernmodell vorherzusagen, das nicht auf Mobilitätsdaten angewiesen ist. Dies könnte dazu beitragen, Risikopatienten automatisch zu erkennen und die Möglichkeit zu schaffen, die körperliche Leistungsfähigkeit von derzeit immobilisierten Patienten objektiv zu bewerten.

Interessenskonflikt Keine

9 Impact of TSG-6 on the healing of critical-size bone defects in mice

Authors Küppers O[1], Bülow J[1], Haffner-Luntzer M[1], Ignatius A[1], Fischer V[1]

Institute **1** Institute of Orthopedic Research and Biomechanics, Ulm University Medical Center, Germany

DOI 10.1055/s-0042-1757529

Introduction Critical-size bone defect is a challenging clinical problem with a high risk of impaired regeneration, which demands novel therapeutic approaches to enhance bone repair [1]. TNF-stimulated gene 6 protein (TSG-6), a protein with anti-inflammatory and pro-regenerative properties, was shown to be secreted by fat-derived mesenchymal stem cells (MSCs) upon inflammatory stimuli and to improve wound healing [2]. Here, we studied whether TSG-6 can stimulate bone regeneration.

Material and Methods To investigate if bone marrow-derived MSCs (BM-MSCs) also produce TSG-6, the cells were primed in vitro for 24h with an inflammation-cocktail (IL-1ß, IL-6, IL-8, TNF, C3a, C5a), mimicking the immune response after fracture, analyzed by qPCR and ELISA. The impact of TSG-6 on bone healing was studied in 12-weeks-old male C57BL/6J mice. A critical-size defect (1.5mm) was created in femur and treated with a collagen gel containing 10µg rhTSG-6. Bone regeneration was analyzed using µCT on day 35 post-surgery.

Results TSG-6 was significantly upregulated in MSCs upon priming among other pro-inflammatory/regenerative genes, including CCL2, VEGFC and COX2. TSG-6 and PGE2 were significantly increased in MSC supernatants upon priming. The dosage of 10µg rhTSG-6 was insufficient to improve bone healing in vivo, as bone volume, tissue mineral density and bony bridging score did not differ compared to untreated controls.

Conclusion Our preliminary data showed that TSG-6 is significantly upregulated in BM-MSCs exposed to an inflammatory microenvironment mimicking the immune response after fracture. However, despite the proven anti-inflammatory/pro-regenerative properties of TSG-6, rhTSG-6 treatment did not improve bone regeneration, possibly due to an ineffective dosage.

Conflict of Interest No Conflict of Interest.

References

[1] Roddy E. et al. Eur J Orthop Surg Traumatol 2018; 28 (3): p. 351–362
[2] Qi Y et al. J Invest Dermatol 2014; 134 (2): p. 526–537

10 Overexpression of cFos leads to a reduced adipose tissue mass independent of osteosarcoma formation

Authors Luther J[1], Baldauf C[1], Neven M[1], Koehne T[1], Rosenthal L[1], Peters S[1], Amling M[1], David JP[1], Schinke T[1]

Institute 1 Department of Osteology and Biomechanics (IOBM), University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20251 Hamburg, Germany

DOI 10.1055/s-0042-1757530

Introduction For several AP-1 transcription factor family members a function in bone and/or adipose tissue metabolism has been described. Ubiquitous overexpression of cFos causes osteosarcoma formation. However, it is unknown whether cFos has a role in fat tissue and whole body metabolism.

Material and Methods We analyze adipose tissue and liver as well as whole body metabolism of mice ubiquitously over-expressing cFos under the H2kb promoter (*cFos*Tg). The results are compared to *cFos*Tg mice lacking Rsk2, that show a reduced osteosarcoma growth.

Results*cFos*Tg mice show an age-dependent reduction of adipose tissue mass, concomitant with a decreased adipocyte diameter. Interestingly, the expression levels of markers for adipocyte differentiation and function are not globally affected in white adipose tissue of these mice. In agreement, *in vitro* differentiation experiments using adipose derived stromal cells show that the cFos induced reduced adipose tissue mass is not caused by a cell-autonomous defect. However, we observed a decreased expression of enzymes regulating lipogenesis in the liver of *cFos*Tg mice as a possible explanation for the phenotype. Most importantly, by analyzing *cFos*Tg;*Rsk2*ko mice that have a reduced tumor growth, we can show that the reduced adipose tissue mass is independent of osteosarcoma formation.

Conclusion We were able to demonstrate that cFos exerts a role in the regulation of adipose tissue metabolism that is independent of its role in osteosarcoma formation.

Conflict of Interest None.

11 Plastin-3 contributes to articular cartilage degeneration

Authors Mählich D[1], Maus I[2, 4], Dreiner M[1], Weyers M[1], Li T[3], Han L[3], Wirth B[2, 4, 5], Niehoff A[1, 6]

Institutes 1 Institute of Biomechanics and Orthopaedics, German Sport University Cologne, Cologne, Germany; 2 Institute of Human Genetics, University of Cologne, Cologne, Germany; 3 School of Biomedical Engineering, Science, and Health Systems, Drexel University, Philadelphia, USA; 4 Center for Molecular Medicine, University of Cologne, Cologne, Germany; 5 Center for Rare Disorders, University Hospital of Cologne, Cologne, Germany; 6 Cologne Center for Musculoskeletal Biomechanics, Medical Faculty, University of Cologne, Cologne, Germany

DOI 10.1055/s-0042-1757531

Introduction Plastin-3 (PLS3) is a ubiquitously expressed actin-binding and -bundling protein which influences F-actin dynamics affecting various cellular processes [1]. Recently it has been found that PLS3 levels in chondrocytes correlate with the degree of cartilage degeneration in human osteoarthritis (OA) [2]. The aim of our study was to characterize the impact of both, Pls3 deficiency (KO) and PLS3 overexpression (OE) on articular cartilage in mice.

Material and Methods We analyzed ubiquitous 6-month-old female *Pls3*KO [3] and *PLS3*OE (homozygous PLS3V5 transgene) mice [4] in comparison to the C57BL/6N (WT) mice (n = 3–8). Mechanical properties of articular cartilage were measured by AFM. Structural degeneration was determined using a modified Osteoarthritis Research Society International (OARSI) score [5]. Immunohistochemical stainings of collagen-type-II and MMP13 were performed. Bone structural parameters were analyzed using µCT. Chondrocytes from newborn mice were isolated to study F-actin structures via immunofluorescence.

Results OARSI score was significantly enhanced ($p < 0.05$) (OE:2.63 ± 1.52; WT:0.42 ± 0.58). Accordingly, the elastic modulus was significantly ($p < 0.05$) decreased (OE:0.305 ± 0.189 MPa; WT:0.909 ± 0.634 MPa). A significantly reduced collagen-type-II staining ($p < 0.01$) associated with an increased MMP13 staining ($p = 0.07$) was detected. The F-actin structure in cells isolated from *PLS3*OE mice showed stress fiber formation at the cell edges compared to WT and *Pls3*KO cells.

Conclusion Articular cartilage homeostasis of 6-month-old female mice is influenced by PLS3 levels.

Conflict of Interest The authors declare that they have no Conflict of Interests.

References

[1] Wolff L et al. Cell Mol Life Sci. 2021; 78 (13): 5275–5301
[2] Mählich D et al. Int J Mol Sci. 2021; 22 (6): 3073
[3] Neugebauer J et al. Hum Mol Gen. 2018; 27 (24): 4249–4262
[4] Ackermann B et al. Hum Mol Genet. 2013; 22 (7): 1328–1347
[5] Glasson SS et al. Osteoarthritis Cartilage 2010; 18: (Suppl 3): S17–S23

12 Establishment of transgenic cell lines with heterozygous mutations in the PTH1 receptor via CRISPR/Cas9

Authors Marnet K[1, 2], Wiesler M[3], Eigenthaler M[3], Herrmann M[1, 2]

Institutes 1 IZKF Group Tissue Regeneration in Musculoskeletal Diseases, Universitätsklinikum Würzburg, Würzburg; 2 Bernhard-Heine-Centrum für Bewegungsforschung, Universität Würzburg, Würzburg; 3 Poliklinik für Kieferorthopädie, Universitätsklinikum Würzburg, Würzburg

DOI 10.1055/s-0042-1757532

Introduction Primary failure of eruption (PFE) is a rare, non-syndromic disease which is characterized by an incomplete tooth eruption and associated with mutations in the parathyroid hormone receptor (PTH1R) [1–3]. However, the cellular background of this disease is not well described. The objective of this study is to establish transgenic cell lines carrying clinical proven mutations to further elucidate the molecular causes and consequences of PFE.

Material and Methods CRISPR/Cas9 was used in a hTERT PDL cell line to establish transgenic cell lines. The sequence of the PTH1R was uploaded into a software which shows all possible guide sequences (gRNA). The gRNA was chosen so that the clinical described mutation was located in the PAM sequence. A HDR template, which is 100 bp long and carries the mutation was designed. The cells were transfected with lipofectamine CRISPRMAX transfection reagent, gRNA, Cas9-Protein and the HDR template for 2 days. Afterwards, single cell colonies were generated to get identical clonal cell lines. Sequencing of the clones was conducted in order to investigate the efficiency of the genome editing.

Results We observed that the CRISPR/Cas9 strategy is working and the transfection efficiency is around 20-40 %. If the generated cell lines carry the desired heterozygous mutations at the exact positions is currently validated.

Conclusion In the future we want to generate more transgenic cell lines and use them to investigate functional consequences of the mutations and test the potential of therapeutic strategies.

Conflict of Interest All authors declare that they have no Conflict of Interest.

References

[1] Subramanian H et al. PLOS ONE. 2016; 11 (11): e0167033
[2] Decker E et al. Am J Hum Genet. 2008; 83 (6): 781–786
[3] Stellzig-Eisenhauer A et al. J Orofac Orthop. 2010; 71 (1): 6–16

13 Gender-specific impact of a pathogenic KDELR2 mutation on survival and lung development

Authors Prohaska A[1], Stephan A[1], Tröder S[2], Etich E[3], Zevnik B[2], Semler O[1], Rehberg M[1], Zaucke F[3]

Institutes **1** Department of Paediatrics, Faculty of Medicine and University Hospital Cologne, University of Cologne; **2** Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne; **3** Dr. Rolf M. Schwiete Research Unit for Osteoarthritis, University Hospital Frankfurt

DOI 10.1055/s-0042-1757533

Introduction To investigate a recessive form of OI which is caused by a mutation in the *KDELR2* gene, a mouse model reflecting the human genotype was generated. The KDELR2 recycles ER-resident proteins from the Golgi back to the ER. Homozygous mice die shortly after birth due to respiratory failure. Additionally, male homozygous animals do not develop a pigment spot in the anorectal region. Thus, the sex of the animals cannot be determined by optical assessment.

Material and Methods The mouse line was generated via CRISPR/Cas gene editing and harbors a cytosine duplication (c.448dupC) which leads to a frameshift. Restriction fragment length polymorphism PCR was used to determine the genotypes and sequences were verified by Sanger sequencing. To investigate respiratory failure, a floating test and histological analysis were performed. To study gender-specific effects on survival and lung morphology, sex was assessed by PCR [1].

Results A total number of 343 animals was analyzed and only 15 % were homozygous mutants, of which 67 % were male. Compared to wildtype the death rate was strongly increased in homozygous mutants (8 % versus 77 %). Lungs from homozygous mutants were sinking in water and therefore most likely were never inflated. H/E staining reveals an arrest of lung development in the pseudoglandular stage.

Conclusion The homozygous KDELR2 mutation affects murine lung development. Even though the death rate is higher in females and the impaired lung development seems to be the cause of death, more detailed studies are needed to show if gender-specific differences exist in the lung phenotype.

Conflict of Interest The authors have no conflicts of interest to declare.

References

[1] Lambert JF et al. Journal of Neuroscience Methods 2000; 95 (2): 127–132

14 Early post-operative exercise promotes bone healing kinetics: *In vivo* study with rats

Authors Suljevic O[1], Schwarze UY[1], Herber V[1], Okutan B[1], Weinberg AM[1], Sommer NG[1]

Institute **1** Department of Orthopedics and Traumatology, Medical University of Graz

DOI 10.1055/s-0042-1757534

Introduction Physical exercise represents a well-known modality for maintaining healthy locomotor mechanism and bones in general [1, 2]. This statement was supported by previous studies which researched the effect of physical exercise on relieving symptoms of osteoporosis, osteoarthritis and other bone-related diseases [3]. Studies which investigated this topic on a preclinical level, demonstrated that treadmill training in rats is a reliable exercise protocol for in depth analysis of bone microstructural changes [4, 5].

Material and Methods Ten Sprague Dawley male rats underwent bi-cortical 1.6 mm hole drilling in both femur diaphysis, after which n = 5 underwent continuous treadmill training over two weeks, while other rats (n = 5) were entitled to sedentary control group. New bone formation labeling was performed by subcutaneous fluorochrome injections at day 0, 14 and week 5. *In vivo* micro CT scans were performed after the surgery and then once a week during the 6-week postoperative period after which euthanasia of all animals was performed. Femur samples were extracted and underwent *ex vivo* scanning and histological evaluation, while serum was used for evaluating bone remodeling markers.

Results Micro-CT data demonstrated increased volume and surface of newly formed bone in defect area of exercise group, as well as enhanced bone healing kinetics. Statistically significant increase was observed after one week in volume of newly formed bone of training in comparison to sedentary group. Alkaline phosphates levels showed increase in both groups, after 6 weeks.

Conclusion Our study demonstrated positive effects of 2-week postoperative treadmill training in terms of enhanced bone healing kinetics and prominent callus formation as confirmed by the radiological methods.

Conflict of Interest None of the authors have any Conflict of Interest to declare.

References

[1] Higuera-Hernández MF et al. Clin Nutr ESPEN 2018; 25: 50–55
[2] Gunter KB et al. Exerc Sport Sci Rev. 2012; 40 (1): 13–21
[3] Faienza MF et al. Int J Environ Res Public Health 2020; 17 (6): 1862
[4] Hao X et al. J Orthop Res. 2021; 39 (6): 1227–1235
[5] Li Z et al. Sci Rep. 2017; 7 (1): 1977

15 Establishment of a protocol to investigate the immunoregulatory properties of BM-MSCs of femoral shaft fracture patients by imitating an proinflammatory environment with TNF-α and/or INF-γ

Authors Übelmesser K[1, 2], Kupczyk E[3], Häusner S[1], Trivanovic D[1, 2], Hölscher-Doht S[3], Herrmann M[1, 2]

Institutes **1** IZKF Group Tissue Regeneration in Musculoskeletal Diseases, University Hospital of Wuerzburg, Wuerzburg; **2** Bernhard-Heine-Center for Locomotion Research, University of Wuerzburg; **3** Department of Trauma, Hand, Plastic and Reconstructive Surgery, University Hospital of Wuerzburg

DOI 10.1055/s-0042-1757535

Introduction Inflammation and the right balance between activation and termination of the immune system on the side of injury plays a role for the healing outcome. Bone marrow mesenchymal stromal cells (BM-MSCs) are known to possess a wide range of immunoregulatory properties, making them interesting for potential clinical applications. Here, we investigate the response of BM-MSCs to a proinflammatory stimulus on gene expression level and their influence on peripheral blood lymphocyte proliferation in a co-culture model.

Material and Methods MSCs were isolated with patient´s consent and ethical commission´s approval from BM of femoral shaft fracture patients, provided with an intramedullary rod. Cells were stimulated for 3 and 24 h with INF-γ and TNF-α. Viability of MSCs was examined with MTT assay. Gene expression analysis of immunomodulatory genes, such as IDO1, VCAM1, COX2, PDL1 was performed with qPCR. Changes in proliferation and phenotypes of PHA-activated lymphocytes were analyzed using flow cytometry after 5 days of co-culture with pretreated MSCs.

Results INF-γ and TNF-α only slightly influenced the viability of MSCs. When co-cultured with pretreated MSCs, PHA-stimulated lymphocyte proliferation was clearly reduced and a tendency towards a shift in the $CD4^+$ and $CD8^+$ populations of PBMCs was observed when co-cultured with stimulated MSCs.

Conclusion Our current findings might indicate that stimulation of MSCs with proinflammatory cytokines increases their immunoregulatory properties. Thus,

the established protocol seems suitable to test the immunomodulatory potential of MSCs from fracture patients and will be employed to investigate potential correlations to the healing outcome.

Conflict of Interest The authors confirm that there is no conflict of interest.

16 Evaluation of the biocompatibility of calcium phosphate-based bone adhesive components

Authors Vasic K[1], Kruse B[2], Böker KO[1], Schilling AF[1], Epple M[2], Lehmann W[1]
Institutes **1** University Medical Center, Clinic for Trauma Surgery, Orthopedics and Plastic Surgery, University of Goettingen; **2** Inorganic Chemistry and Center for Nanointegration Duisburg-Essen (CENIDE), University of Duisburg-Essen
DOI 10.1055/s-0042-1757536

Introduction The traditional approach in treating bone fractures with osteosynthesis uses screws, wires or plates for the connection of bone fragments. This usually requires a follow-up surgery in order to remove metal implants. Biodegradable bone adhesive could replace the usage of the screws and plates and would remove the need for the second surgery. Additionally, it could be used regardless of the bone fragments size and on hard-to-reach areas [1]. The aim of this project is to investigate how a calcium phosphate-based bone adhesive is affecting cell biological processes of bone healing.

Material and Methods LDH assay was used to assess the potential cytotoxicity of the bone adhesive components: Anionic CaP/CMC nanoparticles (CaP – calcium phosphate; CMC – carboxymethyl cellulose) and cationic nanoparticles CaP/CMC/PLL (PLL – poly-L-lysine). The potential cytotoxicity was tested on THP-1 cells, MG-63 and hMSCs at three different time points: 24, 48 and 72 hours.

Results CaP/CMC/PLL nanoparticles had no cytotoxic effect on any of the tested cell lines at low doses. At high doses, CaP/CMC nanoparticles showed a cytotoxic effect (cell viability 40 %) on THP-1 and MG-63 cells after either 48 or 72 hours of incubation. A similar effect was observed with hMSCs, where CaP/CMC had a cytotoxic effect after 48h, but cell viability was restored at the last time point.

Conclusion CaP/CMC/PLL nanoparticles had no cytotoxic effect on the main cell types present in the bone. Further studies will focus on the modification of nanoparticles with a silica shell to enable covalent surface modifications.

Conflict of Interest No Conflict of Interest.

References

[1] Boeker KO et al. Materials. 2019; 12 (23): p. 3975

17 Joint loading by forced running exercise leads to an adaptation of lateral tibia cartilage in COMP deficient mice

Authors Weyers M[1], Mählich D[1], Lorenz C[1], Dreiner M[1], Eckes B[2], Niehoff A[1, 3]
Institutes **1** Institute of Biomechanics and Orthopaedics, German Sport University Cologne, Cologne, Germany; **2** Translational Matrix Biology, Medical Faculty, University of Cologne, Cologne, Germany; **3** Cologne Center for Musculoskeletal Biomechanics, Medical Faculty, University of Cologne, Cologne, Germany
DOI 10.1055/s-0042-1757537

Introduction Although the mechanosensitive cartilage oligomeric matrix protein (COMP) provides adaptor function by connecting different extracellular matrix proteins, COMP deficient ($COMP^{-/-}$) mice have normal skeletal development compared to wildtype littermates [1–3]. This study investigated the effect of forced running exercise on the lateral tibia cartilage (LTC) in $COMP^{-/-}$ mice.

Material and Methods 12-week-old female wildtype C57BL/6N (WT) and $COMP^{-/-}$ mice [3] were assigned into a control (CON) and an exercise (EXE) group (n = 10/group/genotype). EXE group was trained on a treadmill (20 % incline, 20m/min) for 40min/day, 5 days/week for 6 weeks. After the 6 weeks, left hindlimbs were dissected. Bone mineral density (BMD) were measured by µCT. LTC degeneration was evaluated using modified OARSI score [4, 5]. Immunohistochemical staining of matrix metalloproteinase 13 (MMP-13) and matrilin 1-4 was performed.

Results BMD was significantly ($p < 0.01$) reduced in both $COMP^{-/-}$ groups but none of the groups showed LTC degeneration. MMP-13 staining intensity was significantly ($p < 0.01$) reduced in the central region of the LTC in the $COMP^{-/-}$ EXE group. Matrilin 4 staining intensity showed COMP-dependent reduction in the LTC, while in the tibial growth plate (GP) the deficiency of COMP led to reduced levels of matrilin 2–4.

Conclusion This study revealed no exercise-dependent effect on LTC degeneration or BMD in neither $COMP^{-/-}$ nor WT mice. However, exercise-dependent MMP-13 expression was detected in the central region of the LTC in $COMP^{-/-}$ mice indicating altered local catabolic activity. COMP-dependent expression of the matrilins in the LTC and GP identified COMP as an important binding partner of these matrilins.

Conflict of Interest The authors declare that there are no conflicts of interest.

References

[1] Giannoni et al. Biorheology 2003; 40: 101–9
[2] Blumbach et al. Matrix Biol. 2008; 27: 306–318
[3] Svensson et al. Mol Cell Biol. 2002; 22: 4366–4371
[4] Glasson et al. Osteoarthritis Cartilage. 2010; 3: S17–23
[5] Wang et al. Nat Med. 2001; 17: 1674–1679

Namenverzeichnis/Authors' Index

Osteologie 2022; 31: e2–e8