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14–15. November 2014, Wien

Abstracts von Vorträgen und Postern

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Wissenschaftliche Herbsttagung der Österreichischen Gesellschaft für Knochen und Mineralstoffwechsel (ÖGKM) 14.–15. November 2014, Wien

Abstracts von Vorträgen und Postern*

The Calcium-Sensing Receptor: A Tumour Suppressor in the Colon

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Background The classical role of the calcium-sensing receptor (CaSR), a gene which encodes a calcium-binding G protein-coupled receptor, is regulation of calcium homeostasis. CaSR is also expressed in tissues not directly involved in calcium homeostasis like the colon. In colorectal cancer (CRC) we found that CaSR expression is down-regulated, leading to the hypothesis that loss of CaSR provides growth advantage to transformed cells. The aim of the study was to understand the consequences of restoration of CaSR expression.

Methods and Results We stably transfected HT29 CRC cells with the wild-type CaSR ($HT29^{CaSR\text{-}WT}$) or an empty vector as control ($HT29^{EMP}$). Proliferation (assessed by cell counting) of $HT29^{CaSR\text{-}WT}$ significantly decreased compared with the $HT29^{EMP}$ cells whereas apoptotic potential (assessed by Caspase3/7 activity) was increased ($p < 0.001$). These effects were more pronounced when cells were treated with cinacalcet, an allosteric modulator of the CaSR. On treating the cells with the calcilytic NPS 2143, a negative allosteric modulator of the receptor, we could reverse the anti-proliferative, pro-differentiating effects. $HT29^{CaSR\text{-}WT}$ further showed a more epithelial phenotype than $HT29^{EMP}$ cells. Since cancer cells can undergo epithelial to mesenchymal transition (EMT), we investigated expression of a wide spectrum of EMT markers. Expression of mesenchymal markers (alpha-Smooth Muscle Actin and CD90) and EMT-associated transcription factors (Snail, ZEB, and FoxC2) were significantly down-regulated in the $HT29^{CaSR\text{-}WT}$ cells, with a parallel up-regulation in expression of epithelial markers (E-cadherin). Moreover, we could show a reduction in nuclear to cytoplasmic translocation of β-catenin in $HT29^{CaSR\text{-}WT}$ cells compared with $HT29^{EMP}$ cells and that reintroducing the CaSR led to a decreased invasive behaviour of the $HT29^{EMP}$ cells.

To translate these findings *in vivo* we investigated expression levels of markers of proliferation, differentiation and EMT markers in the colon of CaSR/PTH double knockout mice. Animals lacking CaSR had significantly increased expression of proliferation markers, as well as mesenchymal and EMT-associated transcription factors whereas markers of differentiation and epithelial markers were down-regulated ($p < 0.01$). *Ex vivo*, in a cohort of CRC patients, we found significant inverse correlations between CaSR expression and markers of proliferation and EMT, and positive correlations with differentiation markers ($p < 0.001$).

Conclusion Our results demonstrate a tumour suppressive role of CaSR in the colon. Alterations in the expression and function of the CaSR lead to progression of this neoplastic disease by providing growth advantage to cancer cells. Restoration of CaSR expression and function in CRC is linked to regulation of a balance between proliferation, differentiation, and apoptosis and could provide a rationale for novel strategies in CRC therapy.

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Scanning Acoustic Microscopy Reveals Heterogeneity of Mechanical Properties due to Collagen Orientation in Mice Cortical Bone

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The local mechanical properties of bone are influenced not only by the material chemical composition but also by the spatial arrangement of the components, e. g. orientation of collagen matrix. However, not much is known about local elastic modulus variations in cortical bone. Our goal was to use acoustic imaging to map elastic properties of murine bone with a several microns resolution. Rodent long bones exhibit a permanent growth with endosteal/periosteal bone formation and early bone formation residual may remain.

The local microstructure was characterized with a new scanning acoustic microscopy method (SAM-TOF) using tibia from normal mice. Time-of-flight differences of ultrasound pulses across thin transversal cortical sections with known thickness (~ 30 microns) were determined with 0.125 ns time resolution to obtain sound velocity maps (2 µm pixel resolution) using a 330-MHz lens (kibero Gmbh). Velocity maps were combined with density maps derived from calcium content obtained by quantitative backscattered electron imaging to extract dynamic elastic moduli maps. Based on polarized light microscopy, we distinguished bone areas of different collagen fibril arrangement/orientation: bone with predominantly longitudinal collagen orientation (dark; LB), with transverse collagen orientation (bright; TB), and poorly ordered bone (PB) found as an asymmetrically band in the middle of the cortical cross-section.

The mean material density did not differ significantly between the 3 areas. However, the velocity was found significantly lower in TB (–14.5 %) and PB (–12.4 %) compared to LB (4343 m/s). The elastic modulus was found lower in TB (–26 %) and PB (–22 %) compared to LB (33 GPa). No difference between TB and PB was found. No correlation was found between density and velocity ($r^2 = 0.074$) or elastic modulus ($r^2 = 0.055$).

These results show that TOF scanning acoustic microscopy reveals elastic moduli depending on collagen orientation and mineral content and emphasizes the importance of collagen orientation in determining the local mechanical properties of bone.

Vitamin D and Bone Turnover Markers in Normal Pregnancy

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Objective The aim of this study was to investigate the concentrations of vitamin D (25-OH vitamin D) and bone turnover markers in

* Reihung alphabetisch nach Erstautor

normal pregnancy. Based on a previous study where serum levels of OPG, free sRANKL, and DKK-1 were found to be changed significantly between pregnant and non-pregnant women, these markers and vitamin D serum levels where measured within the trimesters of pregnancy, postpartum, and compared to the respective marker serum levels of healthy, non-pregnant women.

In order to develop the fetal skeleton, the mineral metabolism of the mother must adapt to the fetal calcium demand [1]. To which extent maternal bone mineral contributes to the developing skeleton of the fetus is as discussed as the degree to which pregnancy affects changes in long-term bone mineral density (BMD) and the resulting risk of osteoporosis later in life. Some studies characterize pregnancy by high bone turnover with resorption preceding formation [2]. Other groups suggested that OPG and beta crosslaps (CTx) may be involved in the regulation of bone turnover during pregnancy [2, 3] as elevated levels were found in pregnant women. Also significant higher concentrations of sRANKL and OPG were found in the second trimester of normal pregnancy when compared to the first and the third trimester [4]. Maternal vitamin D status during pregnancy is a matter of concern.

Study Design and Results The serum of healthy, pregnant women (n = 125 first trimester T1, n = 86 second trimester T2, n = 3 third trimester T3, and n = 21 postpartum) and of 13 healthy, non-pregnant women about the same age were assayed by ELISA (OPG, DKK1, and free sRANKL by Biomedica, Austria, and vit D by Immundiagnostik AG, Germany). As observed in the previous study, the median OPG serum levels were significantly higher, especially in T1 and T2 (T1 = 4.9, T2 = 6.75, and T3 = 5.25 pmol/l) compared to 3.8 pmol/l for non-pregnant woman (postpartum 5.8 pmol/l), and serum concentrations of DKK-1 significantly decreased particularly in T2 and T3 (T1 = 27.2, T2 = 19.7, and T3 = 21.8 pmol/l) but returning to normal levels (29.9 pmol/l) postpartum (non-pregnant = 29.7 pmol/l). In contrast to our previous study, free sRANKL significantly decreased in all 3 trimesters (T1 = 0.08, T2 & T3 = 0.03 pmol/l). Here again postpartum levels (0.15 pmol/l) were found similar to the levels of non-pregnant women (0.19 pmol/l). These new findings can be explained by the use of a new, more sensitive assay, allowing robust measurements of even low free sRANKL levels. Vitamin D levels decreased during all stages of pregnancy and postpartum (T1 = 40.1, T2 = 45.6, T3 = 30.3, and postpartum = 34.7 nmol/l) compared to non-pregnant women (= 58.8 nmol/l) indicating an undersupply of vit D during pregnancy.

Conclusion Serum levels of OPG, free sRANKL, DKK-1, and vit D were found to be changed significantly between pregnant and non-pregnant women. Significant changes were found mainly in the first 2 trimesters. While the altered levels found for OPG, free sRANKL, and DKK1 may contribute to a prevention of maternal bone loss, the found state of maternal vitamin D deficiency may result in several negative outcomes for mother and child [5, 6]. This indicates the need of an appropriate vitamin D supplementation during pregnancy [7].

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Increased Dickkopf-1 and Follistatin Serum Levels After a 246-km Ultramarathon Race

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Background It is generally accepted that regular physical exercise is beneficial for the skeleton. However, relatively little is known about musculoskeletal effects of extreme forms of exercise. In a previous work [1] we had demonstrated that participation in an ultra-distance race transiently suppressed bone formation and increased bone resorption. The aim of this study was to analyze effects of participation in the Spartathlon, a 246-km ultramarathon race, on novel musculoskeletal markers.

Methods We obtained venous blood samples from 19 participants of the Spartathlon. Samples were taken before and after the race. From 9 participants we additionally obtained blood samples taken one day after the race. We determined serum levels of myostatin (an inhibitor of myogenic differentiation), follistatin (an antagonist of myostatin), and markers/regulators of bone, including the wnt signalling inhibitors sclerostin and dickkopf-1.

Results After the race, a significant 4-fold increase of serum follistatin levels and a slight but significant increase of myostatin levels were observed. Whereas sclerostin levels were not significantly different before and after the race, dickkopf-1 levels significantly decreased by 15 %. Interestingly, one day after the race a significant decrement of sclerostin and dickkopf-1 levels was seen. Serum cathepsin K levels did not differ before and after the ultramarathon.

Conclusion We assume that the marked increase of follistatin and the decline of dickkopf-1 after a 246-km ultramarathon race reflect important anabolic signals for muscle and bone.

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Abnormally High Bone Matrix Mineralization in Children with Osteogenesis Imperfecta Does Not Correlate with Mutation Type or Clinical Severity

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Osteogenesis Imperfecta (OI) is genetically and clinically a heterogeneous disorder characterized by reduced bone mass and increased bone fragility. Most cases of OI are caused by autosomal dominant defects in the genes encoding type-I collagen (OI type I–IV). At the clinical level, OI type I represents the mildest form, which results either from quantitative mutations (due to haploinsufficency in COL1A1 and decreased amount of normal collagen) or from certain qualitative mutations where structurally aberrant collagen chains are generated.

More recently, new recessive forms of OI have been described. Examples are OI type VII, VIII, and IX which are associated with mutations in any of the 3 components of the collagen prolyl-3-hydroxylation complex, responsible for proper collagen processing. Another type is OI VI caused by a mutation in SERPINF1 encoding for pigment epithelium-derived factor (PEDF), a potent antiangiogenic factor. OI type VI has a phenotype with excessive osteoid formation

and prolonged mineralization lag time, suggesting a distinctive mineralization defect.

To get further insight into bone material quality in OI, we evaluated bone mineralization density distribution (BMDD) by quantitative backscattered electron imaging (qBEI) in transiliac biopsies from children (age 2–17.5 years) with different clinical severity and/or different mutation types. Cases include OI type I (quantitative and qualitative mutations), OI type IV, type III, type VII, and type VI. All cases had increased mineral content in the bone matrix. The situation was particularly striking in OI VI, where we found an extremely high mineral content in the bone matrix surrounded by fringes with an unusually low mineral content.

The observation of an abnormally high mineral content in OI bone raises the question whether the increased amount of mineral is due to mineral particles growing to larger sizes or to a higher number of more densely packed particles. To address this question, we evaluated the mineral particle size in 20 biopsy samples by small-angle X-ray scattering (SAXS) and found that the mineral particles are not larger in OI than in controls, suggesting a denser packing of the mineral particles. The case of OI VI was again special in that the mineral particles were even smaller with a rather disordered mineral particle arrangement than in other OI cases and controls. Finally, high-resolution backscattered electron imaging suggests abnormal collagen fibril organization in the perilacunar regions of young osteocytes within the mineralizing matrix.

The Impact of 1,25-Dihydroxyvitamin D_3 on the Wnt Pathway in Colorectal Adenoma Cells

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Epidemiological studies suggest a correlation between vitamin D deficiency and colorectal cancer (CRC) incidence. The majority of sporadic tumours develop from premalignant lesions with aberrant activation of the Wnt/β-catenin signalling pathway. The adenoma cell line LT97 harbours an adenomatous polyposis coli (APC) mutation leading to constitutively active Wnt signalling that can be further transactivated by insulin growth factor 1 (IGF1). In these cells, expression of Wnt target genes leads to increased survival capacity. We hypothesized that 1,25-dihydroyvitamin D_3 (1,25-D_3), the active form of vitamin D_3, promotes differentiation and reduces growth of LT97 cells by modulating β-catenin/TCF (T-cell factor)-4-mediated gene transcription. Furthermore, we investigated the effect of dietary vitamin D on Wnt signalling in a mouse model were we fed mice either with 100 IU or 2500 IU vitamin D/kg diet. We examined the effect of 1,25-D_3 on differentiation by measuring alkaline phosphatase activity and its anti-proliferative potential by cell counting. We analyzed mRNA expression of Wnt target genes by real-time qRT-PCR. The impact of 1,25-D_3 on β-catenin protein expression was assessed by Western blot.

In LT97 cell line, 1,25-D_3 increased cell differentiation, inhibited IGF1-dependent proliferation, and reduced nuclear β-catenin levels. Further, 1,25-D_3 decreased mRNA expression of Wnt target genes BCL-2, Cyclin D1, Snail1, CD44, and LGR5. In healthy colon of mice fed with high vitamin D diet, mRNA levels of Wnt5a and ROR2, that promote degradation of β-catenin, were up-regulated. In conclusion, 1,25-D_3 inhibits Wnt signalling *in vitro* and *in vivo* suggesting a protective role against tumour progression.

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Homocysteine Modulates Mineralization of Osteoblastic Cells

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Hyperhomocysteinemia is associated with several pathologies such as bone fragility, cardiovascular disease, diabetes, and atherosclerosis. We have recently demonstrated that homocysteine (Hcys) alters collagen cross-linking, perturbs triple-helix formation, and regulates expression of genes found in osteoblastic cells, possibly via the inflammation-related gene SAA3. Concerning cardiovascular disease, it was demonstrated that Hcys is related to aortic mineralization in patients with ischemic heart disease. This finding poses the question whether Hcys influences the deposition of mineral in bone cell cultures as well.

For our experiments, we used the pre-osteoblastic cell line MC3T3-E1, which in long-term culture differentiates into mature, mineral-depositing osteoblasts. As a second system we cultured MLO-A5 cells. These cells are late osteoblasts which also deposit mineral, however, already after 10 days.

MC3T3-E1 and MLO-A5 cells were cultured up to 5 and up to 3 weeks, respectively. The cultures were treated either with Hcys or β-glycerophosphate (βGP) or in combination. Thereafter, the cells were fixed with paraformaldehyde and mineralization was measured by Alizarin-red staining. RNA was isolated by an automated isolation system, gene expression was addressed by genome-wide expression analysis (GeneChip, Affymetrix), and expressions of interesting genes were confirmed by RT-qPCR.

Long-term cultures of MC3T3-E1 cells revealed that Hcys in combination with βGP strongly increased the deposition of mineral after 4 and 5 weeks of culture. In MLO-A5 cultures, however, the sole treatment with βGP stimulated the deposition of mineral, and Hcys had no additional effect.

Genome-wide expression analysis and RT-qPCR of Hcys-treated cells demonstrated an increase of Phospho1 (phosphatase, orphan 1) and Alpl (alkaline phosphatase), both genes which are involved in the mineralization process. Interestingly, βGP attenuated the Hcys-stimulated expression of these genes, especially in MLO-A5 cells. This suggests a different mechanism of mineralization in MLO-A5 cells.

Our data suggests that Hcys, by up-regulating expression of phosphatases, increases the concentration of inorganic phosphate which accelerates mineralization of osteoblastic MC3T3-E1 cell cultures. These results also suggest that Hcys can modulate physiological as well as pathological mineralization processes.

Assessment of Microstructure, Bone Mineral Density, and Bone Erosions by HR-pQCT in Psoriatic Arthritis and Psoriasis

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Objective Psoriatic arthritis (PsA) is a chronic inflammatory joint disorder characterized by local bone loss. Additionally, a skin-bone axis in skin psoriasis was suggested recently. However, data on systemic bone loss and bone mineral density (BMD) in PsA as well as in psoriasis are inconsistent. Moreover, data on bone microarchitecture are missing. The aim of this study was to evaluate bone microstructure and volumetric bone mineral density (vBMD) and to de-

Figure 1: Kocijan R, et al. Reconstruction of high-resolution peripheral quantitative computed tomography scans of male patients with **(A)** psoriatic arthritis, **(B)** psoriasis, and **(C)** healthy controls.

tect local bone erosions by HR-pQCT (high-resolution peripheral quantitative computed tomography) in patients with PsA and psoriasis.

Materials and Methods HR-pQCT scans were performed at the periarticular and the non-periarticular radius in patients with PsA (n = 50), patients with psoriasis without any signs of arthritis (n = 30), and healthy, age- and sex-related controls (n = 70). We assessed vBMD and parameters of microstructure including trabecular bone volume (BV/TV), trabecular number (Tb.N), inhomogeneity of the trabecular network, cortical thickness (Ct.Th), and cortical porosity (Ct.Po). Moreover, HR-pQCT scans of the metacarpophalangeal joints (MCP) were performed to detect bone erosions.

Results At the non-periarticular radius, trabecular BMD (p = 0.009), cortical BMD (p = 0.031), BV/TV (p = 0.009), and Tb.N (p = 0.0013) were significantly decreased in PsA patients compared to healthy controls. In addition, Tb.Sp (p = 0.014) and inhomogeneity of the network (p = 0.040) were increased in PsA. Similar results were found at the periarticular radius. No differences were found regarding cortical thickness and cortical porosity between the 3 subgroups.

In contrast, patients with psoriasis without arthritis had a bone phenotype comparable to healthy controls (representative HR-pQCT images are shown in **Figure 1**). Nonetheless, duration of skin disease was found to be associated to low BV/TV and Tb.N in patients with PsA. Bone erosions were detected in 70 % of patients with PsA, 47 % of patients with psoriasis, and 30 % of healthy controls.

Conclusion Trabecular and cortical vBMD as well as trabecular bone microstructure are significantly decreased in PsA. Our data suggest a skin-bone axis linked to duration of psoriasis and a pre-PsA status in patients with psoriasis without arthritis. However, systemic bone loss seems to be triggered by additional factors linked to arthritis.

Knochengesundheit bei dialysepflichtiger Niereninsuffizienz

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Hintergrund Die chronische Niereninsuffizienz ist ein weltweites Gesundheitsproblem. Häufige Komplikationen sind die Störung des Mineral- und Knochenstoffwechsels mit einem hohen Frakturrisiko sowie eine erhöhte Prävalenz kardiovaskulärer Erkrankungen. Ziel der aktuellen Studie ist die Analyse des Knochenstoffwechsels und der Knochendichte an 3 verschiedenen Messpunkten bei Dialysepatienten in Hinblick auf deren Aussagekraft hinsichtlich Frakturprävalenz und Schmerzen.

Methoden In die Studie wurden 72 Dialysepatienten (44 Männer, 28 Frauen) des LKH-Universitätsklinikums Graz eingeschlossen. 47 haben eine Peritonealdialyse, 25 sind an der Hämodialyse. Das Durchschnittsalter beträgt 58 ± 15 Jahre, die mittlere Gesamtdialysezeit liegt bei 46 ± 40 Monaten. Die Knochendichte wurde mittels Dual-Röntgen-Absorptiometrie (DXA, Lunar Prodigy GE) an der Lendenwirbelsäule, der Hüfte und dem nicht dominanten Radius gemessen und es wurden biometrische Daten wie Alter, Geschlecht, Dialysedauer, Nierentransplantationen und Begleitmedikation erhoben. Die Laboranalyse erfasste u. a. die Serumkonzentrationen von Gesamtkalzium, freiem Kalzium, Phosphat, Parathormon, alkalischer Phosphatase und 25OH-Vitamin D. Alle Patienten wurden bezüglich Frakturen und Knochenschmerzen befragt.

Ergebnisse Die Frakturprävalenz in diesem Patientenkollektiv beträgt 22,2 % (n = 16; Wirbelkörperfrakturen n = 2). Sie steht mit niedrigen Knochendichtewerten (Z-Score) am ultradistalen Radius (p = 0,006) und am gesamten Radius (p = 0,022) im Zusammenhang. Gemäß der Knochendichtemessung am ultradistalen Radius haben 52,3 % der Dialysepatienten eine Osteoporose. Die Knochendichte ist negativ mit der Dialysedauer (p = 0,005), dem Alter (p = 0,009), dem PTH-Spiegel (p = 0,013) und der AP-Konzentration (p = 0,007) assoziiert sowie positiv mit dem BMI (p = 0,000). Knapp ein Drittel der Studienteilnehmer (29,2 %) hat Schmerzen, die zwar nicht mit der Frakturprävalenz korrelieren, jedoch mit der Knochendichte am ultradistalen Radius (p = 0,004) und am gesamten Radius (p = 0,003) signifikant negativ assoziiert sind.

Conclusio In unserem Kollektiv eignet sich nur die Knochendichtemessung am Radius zur Bestimmung des Frakturrisikos von Dialysepatienten. Knochenschmerzen sind mit niedrigen Knochendichtewerten am Radius assoziiert und können somit als frühes Warnzeichen für den Knochenverlust interpretiert werden.

Sclerostin Levels and Severe Changes in Bone Metabolism After Bariatric Surgery

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Context The role of sclerostin (Scl), as a key regulator of bone formation, remains unknown after Roux-en-Y gastric bypass (RYGB) or laparoscopic sleeve gastrectomy (SG).

Objectives Evaluation of sclerostin serum levels after surgery and correlations with bone turnover markers and areal bone mineral density (BMD) changes.

Design and Setting A prospective observational single-centre 2-arm study in premenopausal women with morbid adipositas over 24 months.

Participants 52 premenopausal obese women (40 ± 8 years, BMI 43.4) after RYGB and 38 premenopausal women (41 ± 7 years, BMI 45.7) after SG.

Main Outcome Measures Prior to surgery and after 1, 3, 6, 9, 12, 18, and 24 months, Scl, CTX, P1NP, and BMD were measured.

Results Scl increased by Δ +50.5 % at 30 ± 5 days after surgery and remained elevated with a maximum of Δ +120 % 6 months after surgery. CTX rapidly and continuously increased by Δ +170 % (peak month 9 Δ +190 %), P1NP to lesser extend (peak Δ +73 % at

month 12), and steadily declined to a change of Δ +36 % ($P < 0.001$ for all). Scl increases were significantly positively correlated with CTX and P1NP increases and significantly negatively correlated with BMD loss. BMD independently declined regardless of RYGB and SG. Elevations of Scl, CTX, P1NP, and phosphate ($p < 0.001$), but not iPTH, were significant discriminating factors for BMD loss (AUC 0.915).

Conclusion Rapid and sustained increase of Scl, CTX, and to lesser extend P1NP causes an uncoupling in bone metabolism and results in BMD loss at all skeletal sites.

Characterization of Bone Tissue Using a Combination of Scanning Electron Microscopy and Confocal Laser Scanning Microscopy

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Quantitative backscattered electron imaging (qBEI) used in a scanning electron microscope (SEM) is a powerful tool for 2-dimensional visualization and quantification of the mineralized part of bone tissue at the microscopic level. SEM also allows analyzing the local chemical composition of the bone matrix using energy dispersive X-ray analysis (EDX). Despite the power of these techniques, information on the micro-architecture (structure of the osteocyte lacunae canaliculi network [OLCN]) and tissue age is missing.

To overcome these restrictions, we combined SEM (qBEI and EDX) on identical sample surfaces with confocal laser-scanning microscopy (CLSM) providing surface and depth scans of bulk bone tissue after staining with Rhodamine-6G or fluorochrome labelling. Rhodamine-6G is a highly efficient fluorescent dye which accumulates in the non-mineralized organic matrix of bone. It has been introduced into bone research, especially to visualize the 3-dimensional structure of OLCN, which is suspected to play a major role in mechanosensing and regulation of the bone metabolism. Thus the combination of qBEI, EDX, and CLSM allows studying the relationship between tissue composition (mineral content, tissue age) and OLCN parameters. Further, fluorochrome-labelling techniques allow normalizing results from chemical and morphological analysis for tissue age, providing additional information on the time course of bone development.

qBEI, EDX, and CLSM are compatible, non-destructive methods to analyze identical (and if desired tissue age-normalized) regions of embedded bone. Hence, comprehensive information on the chemical composition of the surface in relation to the canaliculi network structure can be provided.

Novel Highly Sensitive ELISA to Measure Free, Bioactive, Human Soluble RANKL

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RANKL, the receptor activator of nuclear factor kappa-B ligand, together with its receptor RANK and the antagonist Osteoprotegerin (OPG) is a key regulator in bone metabolism, namely in the formation of mature osteoclasts [1]. Bioactivity of RANKL has only been reported for the soluble form (sRANKL), which is generated from the membrane-bound protein RANKL [2]. sRANKL can circulate either bound to OPG or in an even lower amount as free sRANKL. Compared to whole sRANKL (bound and unbound form), free sRANKL has been proven difficult to measure: the accuracy of sRANKL measurement is compromised by the very low or undetectable levels, as observed in some patient cohorts [3].

Hence, our aim was to develop a highly sensitive and specific assay that enables the direct measurement of free, bioactive, soluble RANKL in serum and plasma samples. We have taken advantage of the high affinity and specific protein-protein interaction between sRANKL and OPG and used immobilized, recombinant OPG to capture free sRANKL, which subsequently is detected with a biotin-labelled anti-sRANKL antibody.

The data presented here demonstrate that all samples (n = 127) from an unselected clinical and 2 apparently healthy populations had detectable free sRANKL values within the calibration range of the assay (0–2 pmol/l). The median of serum samples, prepared immediately after blood collection and stored at –25 °C until measurement, was 0.14 pmol/l or 0.16 pmol/l, respectively, with a lower limit of quantification (LLOQ) of 0.01 pmol/l. Assay characteristics, such as spike/recovery, dilution linearity, and analyte stability in whole blood as well as intra-/inter-assay precision have been analysed.

Our novel ELISA provides a reliable and accurate tool for the quantitative determination of free, soluble, bioactive RANKL in human samples.

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Downstream Signalling of The Calcium-Sensing Receptor in Colon Cancer Cells

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Background The calcium-sensing receptor (CaSR), a G protein-coupled receptor, which is able to sense changes in extracellular calcium levels, regulates various cellular functions such as cell proliferation and survival. During colorectal tumourigenesis, CaSR expression is down-regulated. This led us to hypothesize a deregulation of CaSR-mediated signalling in the colon. Therefore, the aim of this study is to investigate the signalling pathways regulated by the CaSR in colonocytes.

Methods Live cell imaging with Fura-2 fluorescent dye was performed to determine the intracellular calcium levels. Calcium-mediated phosphorylation of ERK1/2 and p38 was determined by either traditional or in-cell western blotting in the colon cancer cell line Caco2-15 which expresses endogenous CaSR. Involvement of receptor was confirmed with specific allosteric modulators of the CaSR (agonist NPSR-568 and antagonist NPS2143). Further, the role of the receptor was investigated on stably transfected HT29 cells (which do not express the CaSR) over-expressing the wild-type CaSR (CaSR-WT). The cells transfected with the empty vector (Emp) was used as control.

Results Increasing concentrations of extracellular calcium increased intracellular calcium levels in Caco2-15 cells. However, in HT29 cells, which do not express the receptor, extracellular calcium was unable to evoke any intracellular calcium response, whereas re-introduction of the CaSR into these cells was able to induce striking calcium oscillations.

In Caco2-15 cells a 10-minute treatment with calcium showed a significant down-regulation of ERK1/2 phosphorylation while the phosphorylation of p38 was inhibited. The positive allosteric modulator NPS R-568 further enhanced the calcium-mediated signalling which was abrogated in the presence of the negative modulator NPS 2143.

Conclusions We have shown that the CaSR induces extracellular calcium-mediated intracellular calcium levels in colon cancer cells. Whether this is achieved by opening the cell-membrane channels or intracellular stores needs to be proven. The involvement of the CaSR in regulation of the MAPK pathway could be linked to the cancer-preventive effects of calcium.

Gender Differences in the Biochemical Profile of Primary Hyperparathyroidism – the EPATH Study

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Introduction Previous studies in healthy populations indicated gender-specific effects of parathyroid hormone (PTH) on individual calcium levels. However, data on patients with primary hyperparathyroidism (pHPT) are lacking. Therefore, we aimed to assess whether calcium or phosphate levels and their correlation with PTH reveal gender-specific differences in pHPT patients.

Methods We analysed data of pHPT patients who participated in the EPATH study ("Effects of Eplerenone on Parathyroid Hormone Levels in Patients with Primary Hyperparathyroidism"). Assessment of anthropometric data and blood sampling were performed between 8 am and 12 am after an overnight fast. PHPT was defined as either plasma calcium > 2.55 mmol/L and plasma PTH > 46 pg/mL or as calcium > 2.40 mmol/L and PTH > 65 pg/mL according to current international guidelines. Patients with regular use of cinacalcet were excluded from the analysis.

Results The study population comprised 127 pHPT patients including 101 postmenopausal women (80 %) and 26 males. The mean age was 68 ± 9 years, median PTH was 95 pg/mL (IQR = 79–116), mean calcium was 2.62 ± 0.13 mmol/L, mean phosphate was 2.54 ± 0.4 mg/dL, and median 25-hydroxyvitamin D_3 (25[OH]D) was 33 ng/mL (IQR 25–42). Hypercalcemia (calcium > 2.55 mmol/L) was present in 62 females (62 %) and in 21 males (81 %). PTH did not differ significantly between genders, while calcium levels were higher and phosphate levels were lower in males (2.69 ± 0.16 mmol/L vs 2.61 ± 0.12 mmol/L, P = 0.004; 2.22 ± 0.5 mg/dL vs 2.51 ± 0.3 mg/dL, P = 0.001). This remained significant after adjustment for age, PTH, 25[OH]D, estimated glomerular filtration rate (eGFR CKDEPI), BMI, and thiazide diuretic use (P = 0.006 and P = 0.001, respectively). PTH was stronger correlated with calcium and phosphate among males than females, even after adjusting for 25[OH]D (calcium: r = 0.571 vs r = 0.205, P < 0.05 for each; phosphate: r = –0.458 vs –0.355, P < 0.05 for each). Among hypercalcemic pHPT patients, PTH correlated with calcium only in males when adjusted for 25[OH]D (r = 0.547, P = 0.013 vs r = 0.071, P = 0.59).

Conclusion In a large cohort of pHPT patients, males had higher calcium and lower phosphate levels, even after adjustment for potentially confounding parameters. These data strengthen the notion that PTH effects on calcium metabolism may underlie gender-specific regulatory mechanisms. Males may be more responsive to high PTH levels and may thus be predisposed to more severe forms of pHPT.

Donor Age-Dependent Influence of Circulating Extracellular Vesicles on In Vitro Bone Formation

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Aging is a complex process resulting in the decline of physiologic functions as well as in reduced repair capacities. The regenerative power of stem cells has been found to decline with age and to be influenced by the systemic environment. In particular, osteogenic commitment of mesenchymal stem cells (MSCs) has been shown to decrease with age thereby resulting in slowed down bone formation that contributes to osteoporosis. Here we set out to identify circulating factors of the aged systemic environment that influence the functionality of adult stem cells.

In order to identify such factors, the influence of extracellular vesicles (EVs) isolated from the plasma of young versus elderly healthy individuals on the osteogenic differentiation process was tested. Functional characterization of EVs revealed that EVs of elderly donors inhibit osteogenesis compared to young plasma derived vesicles. When searching for differences in the miRNA and protein content, anti-osteogenic miR-31 was found to be higher in elderly derived vesicles while pro-osteogenic Galectin-3 was significantly decreased in the plasma of elderly donors. Therefore, we postulate here that circulating EVs and their content might represent therapeutic targets and biomarkers for a systemic environment that does not facilitate bone formation, especially in age-related diseases like osteoporosis.

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