The polyphenol fisetin protects bone by repressing NF-kB and MKP-1-dependent signaling pathways in osteoclasts

Laurent L. Leotoing, Fabien F. Wauquier, Jérome Guicheux, Elisabeth Miot-Noirault, Y. Wittrant, Véronique Coxam

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Programme

1er jour – 04 février 2015

Mise en place de la navette Clermont gare / Aéroport / Super Besse
(1 seule navette : départ 11h de la gare puis direction aéroport puis Super Besse)
Arrivée à Super Besse vers 12h30.
Les bagages seront déposés en salle plénière.

12h45 déjeuner sur place

14h00 allocution de bienvenue

14h15 – 16h15 Session 1 *modérée par Edith Bonnelye et Arnaud Bianchi*

Conférence plénière de 45' accompagnée 15' de discussion
Biomineralization and biomechanic of bone
Georges Boivin, DR INSERM 1033, Université de Lyon, France

Présentations orales de 10' avec 5' de questions
1. Muscle injury impairs bone regeneration in adult mice. Julien Anais
2. Pit1 mediates survival of chondrocytes from endoplasmic reticulum-induced stress in vivo. Greig Couasnay
3. miR-199a/b-5p and Runx2 collaborate to regulate Wnt/β-catenin pathway. Mylène Zarka
4. IIb or not IIb: development of an original screening method by flow cytometry to characterize diverse sources of human mesenchymal stem cells and evaluate their potential for cartilage reconstruction. Hugo Fabre

16h15 - 16h45 pause et remise des clés des chambres (réception Belambra ; noms de A à L)

16h45 - 18h45 Session 2 *modérée par Catherine Le Visage et Sylvie Tambutté*

Conférence plénière de 45' accompagnée 15' de discussion
Chimie douce, nanostructures minérales et biologie
Jacques Livage, Professeur honoraire au collège de France, Paris, France

Présentations orales de 10' avec 5' de questions

5. The european abalone (Haliotis Tuberculata) shell: a relevant model to study the impact of ocean acidification. Stéphanie Auzoux-Bordenave
6. Mineralization of the mouse trachea is a sudden and early physiological event. Chaohua Deng
7. FGF-23 regulates MMP-13 through FGFR-1 in human Osteoarthritis chondrocytes via PI-3K and ERK pathways. Mathilde Guibert
8. In vivo measurement of pH at the sites of calcification of the red coral corallium rubrum. Le Goff Carine

18H45 Remise des clés des chambres (réception du Belambra ; noms de M à Z)

20h dîner buffet

21h – 00h Flash posters, fromages et vins (salle plénière)
Séance modérée par Reine Bareille et Véronique Coxam
26 posters : 3’ par poster
26 posters en diaporama continu
Discussions autour de vins et fromages (avec modération…)

2ème jour – 05 février 2015

7h30 – 9h petit déjeuner sur place
7h30 – 9h petits déjeuners thématiques

9h - 13h Session 3 *modérée par Jérôme Guicheux et Yohann Wittrant*

Conférence plénière de 45' accompagnée 15' de discussion
Long chain polyunsaturated fatty acids and bone health
Marlena Kruger, Professor of nutritional physiology, Massey University, New Zealand

Présentations orales de 10' avec 5' de questions

9. Effects of Osteum, a natural ingredient, containing micellar calcium, vitamin D and K2, on bone mineral density. Anne Blais
10. Osteoclast activity regulates hematopoietic stem cell niches during inflammatory bowel disease. Agathe Boucoiran
11. Deficiency in claudin-16 tight junction protein as novel cause of Amelogenesis Imperfecta. Claire Bardet
12. The polyphenol fisetin protects bone by repressing NF-kB and MKP-1-dependent signaling pathways in osteoclasts. Laurent Léotoing

11h -11h30 pause

11h30 - 12h30 Session 4 *modérée par Said Kamel et Pierre Hardouin*

4 présentations orales de 10' avec 5' de questions

13. HIF signaling in skeletal progenitors promotes breast cancer growth and metastasis through systemic production of CXCL12. Claire-Sophie Devignes
14. Osteoclasts expressing cx3cr1 induce tnfα-producing cd4+ t cells in inflammatory condition. Lidia Ibáñez
15. Calpain-6 expression identifies a stem cell population in osteosarcoma. Caroline Andrique
16. miR-146a deficiency in Ly6Chigh monocytes contributes to pathogenic bone loss during inflammatory arthritis. Meryem Ammari

12h30 – 13h30 déjeuner buffet sur place.

13h30 – 17h Activité de groupe (deux groupes de 75 pers. alternant)
1h30 de Randonnée raquette puis 1h30 Construction d'igloo / Initiation ARVA (secours en

cas d'avalanche)

17h30 – 18h pause (café, chocolat et vin chaud)

18h - 19h Session 5 *Modérée par Claudine Blin et Cécile Colnot*

Conférence plénière de 45' accompagnée 15' de discussion
Macrophages and bm stem cell niches: the roles of pge2 and coagulation.
Tsvee Lapidot, Professor, Department of Immunology, Weizmann Institute, Israel

19h – 20h Assemblée générale (salle plénière)

20h Apéritif
20h30 Dîner de gala
22h Soirée dansante

3ème jour – 06 février 2015

7h30 – 9h petit déjeuner sur place

9h - 10h30 Session 6 *modérée par Alain Guignandon et Laurent Beck*

Conférence plénière de 45' accompagnée 15' de discussion
Réponses adaptatives du squelette à la contrainte mécanique : rôle de la périostine
Nicolas Bonnet, Geneva University hospital & faculty of medicine, Switzerland

2 présentations orales de 10' avec 5' de questions

17. Rôle fonctionnel du récepteur à l'acide lysophosphatidique (LPA1) au cours de la différentiation ostéoclastique et de la résorption osseuse. Irma Machuca-Gayet
18. Primary cilia of the cartilage is abnormal in FGFR3-related disorders. Ludovic Martin

10h30 -11h pause

11h remise des prix

11h Libérations des chambres et remise des clés à la réception du Belambra

12h15 départ première navette pour clermont gare / clermont aéroport

12h - 14h déjeuner sur place.

14h départ de la seconde navette pour clermont gare / clermont aéroport

Fin des jfbtm 2015…

Le congrès s'effectue sur 3 journées, il n'est pas prévu de navettes intermédiaires pour effectuer la liaison Clermont-Ferrand / Super-Besse en dehors du mercredi et du vendredi.

Liste des communications orales par ordre de passage

Liste des présentations orales "classiques" dans l'odre de passage

Titre du Résumé	Auteurs et Orateurs	Session	Journée	Etudiant
Muscle injury impairs bone regeneration in adult mice	Julien Anais	1	Mercredi après midi	X
PiT1 mediates survival of chondrocytes from endoplasmic reticulum-induced stress in vivo	Greig Couasnay	1	Mercredi après midi	X
miR-199a/b-5p and Runx2 collaborate to regulate Wnt/β-catenin pathway	Mylène Zarka	1	Mercredi après midi	X
IIB OR NOT IIB: DEVELOPMENT OF AN ORIGINAL SCREENING METHOD BY FLOW CYTOMETRY TO CHARACTERIZE HUMAN MESENCHYMAL STEM CELLS...	Hugo Fabre	1	Mercredi après midi	X
THE EUROPEAN ABALONE (HALIOTIS TUBERCULATA) SHELL: A RELEVANT MODEL TO STUDY THE IMPACT OF OCEAN ACIDIFICATION	Stéphanie AUZOUX-BORDENAVE	2	Mercredi après midi	
MINERALIZATION OF THE MOUSE TRACHEA IS A SUDDEN AND EARLY PHYSIOLOGICAL EVENT	Chaohua DENG	2	Mercredi après midi	X
FGF23 REGULATES MMP13 THROUGH FGFR1 IN HUMAN OA CHONDROCYTES VIA PI-3K AND ERK PATHWAYS	Mathilde GUIBERT	2	Mercredi après midi	X
IN VIVO MEASUREMENT OF PH AT THE SITES OF CALCIFICATION OF THE RED CORAL CORALLIUM RUBRUM	LE GOFF Carine	2	Mercredi après midi	X
Effects of Osteum, a natural ingredient, containing micellar calcium, vitamin D and K2, on bone mineral density	Anne Blais	3	Jeudi matin	
Osteoclast activity regulates hematopoietic stem cell niches during inflammatory bowel disease	Agathe BOUCOIRAN	3	Jeudi matin	X
Deficiency in claudin-16 tight junction protein as novel cause of Amelogenesis Imperfecta	Claire Bardet	3	Jeudi matin	
The polyphenol fisetin protects bone by repressing NF-kB and MKP-1-dependent signaling pathways in osteoclasts	Laurent Léotoing	3	Jeudi matin	

HIF signaling in skeletal progenitors promotes breast cancer growth and metastasis through systemic production of CXCL12	Claire-Sophie Devignes	4	Jeudi matin	X
OSTEOCLASTS EXPRESSING CX3CR1 INDUCE TNFα-PRODUCING CD4+ T CELLS IN INFLAMMATORY CONDITION	Lidia Ibáñez	4	Jeudi matin	
CALPAIN-6 EXPRESSION IDENTIFIES A STEM CELL POPULATION IN OSTEOSARCOMA	Caroline Andrique	4	Jeudi matin	X
miR-146a deficiency in Ly6Chigh monocytes contributes to pathogenic bone loss during inflammatory arthritis	Meryem AMMARI	4	Jeudi matin	X
Rôle fonctionnel du récepteur à l?acide lysophosphatidique (LPA1) au cours de la différentiation ostéoclastique et de la résorption osseuse.	Irma Machuca-Gayet	6	Vendredi matin	
Primary cilia of the cartilage is abnormal in FGFR3-related disorders	Ludovic MARTIN	6	Vendredi matin	

Liste des communications rapides (flash posters) par ordre de passage
(+/- ordre alphabétique des prénoms)

Liste des posters dans l'ordre de présentation

INVESTIGATION OF THE CALCIUM-SENSING RECEPTOR EXPRESSION IN MONOCYTES ISOLATED FROM SYNOVIAL FLUIDS	Alice Séjourné	P1
HYPOXIA-DEPENDENT DNA METHYLATION TO CONTROL THE CHONDROCYTE PHENOTYPE	Anne-Laure Durand	P2
A NEW ALTERNATIVE IN BONE REGENERATION: COMBINATION OF CHITOSAN/HYDROXYAPATITE SCAFFOLD, NACRE ACTIVE COMPOUNDS AND STEM CELLS	Anne-Sophie WILLEMIN	P3
HYDROLYZED COLLAGEN PROMOTES OSTEOBLASTOGENESIS AND PRESERVES BONE MASS IN OVARIECTOMIZED MICE	audrey daneault	P4
THE ESTABLISHMENT OF MICROFLUIDIC CO-CULTURE SYSTEM TO STUDY THE INTERPLAY BETWEEN SENSORIAL NERVOUS SYSTEM AND MESENCHYMAL STEM CELLS IN VIEW OF OSTEOGENESIS	BRUNO PAIVA DOS SANTOS	P5
Bioingeneering of mandibulare reconstruction in cancer surgery	Camille EHRET	P6
The flavonoid fisetin promotes osteoblasts differentiation through Runx2 transcriptional activity	Cédric Darie	P7
OSTEOCLASTS ACTIVATE CHONDROCYTE CATABOLISM THROUGH S1P PRODUCTION.	CHAHRAZAD CHERIFI	P8
SILICON IN VEGETABLES: IN VITRO BIOACCESSIBILITY, BIOAVAILABILITY AND BIOLOGICAL ACTIVITY IN DIFFERENT TARGET TISSUES (INTESTINE AND BONE)	D'Imperio Massimiliano	P9
EFFECT OF THE LEUCINE RICH AMELOGENIN PEPTIDE ON ENAMEL STRUCTURE AND MINERALIZATION	Elvire Le Norcy	P10
SEPARATION AND IDENTIFICATION OF THE OSTEOGENIC COMPOUNDS OF NACRE USING ION-EXCHANGE RESIN ASSOCIATED WITH A MINERALIZATION CELL MODEL	Ganggang ZHANG	P11
Bone loss in early phases of rat arthritis is predictive to disease severity	Guillaume COURBON	P12
A new therapeutic approach for achondroplasia and hypochondroplasia: Tyrosine Kinase Inhibitor BN016	KOMLA-EBRI Davide	P13
DEVELOPPEMENT D'UN MODELE D'OSTEOGENESE IN VITRO AU SEIN DE MATERIAUX 3D	Laura JUIGNET	P14
DISTINCT EXPRESSION OF IL-36 AND THEIR ANTAGONISTS IN RHEUMATOID ARTHRITIS	Marie-Astrid Boutet	P15
CYSTIC FIBROSIS BONE DISEASE: AN ELEVATED RANK-L/OPG PROTEIN RATIO IN OSTEOBLAST WITH THE F508DEL-CFTR MUTATION	Martial Delion	P16
Glycosyl-Nucleosyl-Fluorinated - Collagen injectable hydrogel for tissue engineering: A new scaffold for bone regeneration.	Mathieu Maisani	P17
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GENERATION OF NUCLEUS PULPOSUS PROGENITOR CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS.	Pauline Colombier	P20
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A NEW ES CELL LINE AS AN ALTERNATIVE SOURCE FOR NEURAL CREST DERIVED STRUCTURES IN TISSUE ENGINEERING.	Soledad ACUNA MENDOZA	P22
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NAMPT IS REQUIRED FOR OSTEOBLASTS ACTIVITY AND OSTEOCLASTOGENESIS IN BONE REMODELING	Bassam HASSAN	P27
Phosphate transporters, functional implication in dental mineralization	Laure MERAMETDJIAN	P42
Human Amniotic Membrane for osteoregeneration of calvaria defects	Mathilde FENELON	P44
Dentin repair in sost ko mice: following of dentin reactions in a model of pulp exposure	Anne-Margaux Collignon	P25
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Reinforcements of Si-HPMC hydrogel for cartilage engineering	Cécile Boyer	P29
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DENTAL PULP REGENERATION IN MINI-PIG	francesca mangione	P34
RUNX2 AND VDR EXPRESSION IN THE CONTROL OF OSTEOBLAST FUNCTION AND BONE FORMATION	Frédéric Jehan	P35
BONE DISEASE IN CYSTIC FIBROSIS : IMPAIRMENT OF MATURATION KEY FACTORS EXPRESSION IN OSTEOBLASTS AND POTENTIAL THERAPEUTIC	Frédéric VELARD	P36
Potential of dental pulp stem cells for bone defect regeneration	Gael Rochefort	P37
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ASSOCIATION D'UN LYSAT DE MOELLE OSSEUSE ET DE BIOMATÉRIAU PHOSPHOCALCIQUE DANS LA RECONSTRUCTION DE L'OS IRRADIÉ	Guillaume MICHEL	P40
Pannexin 1 and pannexin 3 regulate osteoblastic differentiation of human bone marrow mesenchymal stem cells within a three-dimensional macroporous scaffold.	Julien Guerrero or Hugo Oliveira or Joëlle Amédée	P41
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Imagerie in vivo de la vascularisation par micro-scanner	Sadoine Jérémy	P46
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Mesenchymal stem cell differentiation towards osteoblast using 3D culture models.	valérie TRICHET	P51
TRABECULAR BONE ARCHITECTURE: ITS PIVOTAL ROLE IN BONE FRAGILITY	Yohann BALA	P52

BIOMINERALIZATION AND BIOMECHANIC OF BONE

Georges BOIVIN

INSERM UMR 1033, Université de Lyon, Lyon, France

Résumé

Involved in locomotion, stature, protection of organs and as reservoir of ions, bone is a living material having a hierarchical structure (Bala et al. 2013, Osteoporos Int 24:2153-66). At bone level, cortical surrounds cancellous tissue (network of trabeculae) and bone marrow. Bone structures are composed of a lamellar texture in adult following a transitory woven texture during growth. In human, after the modeling phase, almost each bone is submitted to a permanent remodeling. At tissue level, bone is composed of bone structural units (osteons in cortical and bone packets in cancellous) corresponding to the net production of bone tissue following a remodeling cycle. Bone tissue is composed of a mineral phase (biological apatite crystals) in an organic matrix (mainly type I collagen fibrils). Mineralization involves not only the initial deposition (heterogeneous nucleation then secondary nucleation) of mineral in an organic matrix but also its maturation until the upper mineral density in a given volume of matrix is reached. Independently of bone mass and its distribution in space (geometry/architecture), the mineralization and the “quality” of the mineral play a crucial role in the elastic, plastic, and viscoelastic properties defining the mechanical behavior of bones. The mineral phase stiffens the bone, while the heterogeneity of mineralization defines its ability to resist to crack propagation. At the nanoscopic scale, the “quality” of mineral crystals deposited in the collagen frame is a key factor in the mineralization process and independently predicts bone mechanical properties, at either local or global levels. The presence of apatite enhances the tensile modulus and strength of collagen. Conversely, organic matrix directly acts on the proportions of loads transferred on mineral particles preventing mineral cracking. Longer crystals may reflect larger surface area of interaction that may affect the mobility of collagen molecules, decreasing tissue ductility (i.e., the ability of the material to deform plastically without breaking). Regardless of crystal size, the nature of the interface between mineral and collagen itself has a determinant role in bone mechanical behavior. Individually, both apatite and collagen fibrils have a mostly elastic mechanical behavior. However, bone tissue formed with those two components has a visco-elasto-plastic mechanical behavior. Recent findings have highlighted the need to understand the underlying process occurring at nanostructural level that may be independent of bone remodeling itself. Acknowledgments. Author expresses its gratitude to Delphine Farlay, Yohann Bala and Audrey Doublier (INSERM 1033, Lyon, France) for their expert collaboration and fruitful discussions.

Mots-clés

Bone - Mineralization - Biomechanic - Quality of Mineral - Apatite Crystals

Muscle injury impairs bone regeneration in adult mice - O1

Julien Anais and Colnot Celine
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Résumé

Bone and muscle exhibit great capacities to regenerate after injury [1,2]. However, delayed union or non-union occur in 5 to 10% of all fractures and is increased up to 46% in patients with extreme trauma and soft tissue damage such as skeletal muscle. Muscle flaps are used clinically to improve bone repair, and the role muscle in bone repair is well recognized but the mechanisms are poorly understood. Recent data from the laboratory have shown that muscle is a source of chondrocytes and that muscle stem cells, the satellite cells, are functionally involved in bone repair acting as a source of growth factors including Bone Morphogenetic Proteins (BMPs) [3,4]. The BMP pathway is largely involved in bone and muscle development, growth and repair [5]. BMP receptor 1A (BMPR1A) is the major receptor of BMP ligands involved in bone growth and regeneration. In this context, we hypothesized that severe muscle injury impairs bone regeneration and perturbs the normal bone-muscle interactions mediated by BMP signaling. To mimic musculoskeletal traumatic injury, we established a novel model of crush muscle injury in mice in order to determine the effects of crush muscle injury on fracture repair. Protocols were approved by the Paris Descartes University Ethical committee. We induced muscle crush injury in three-month old mice and analyzed muscle regeneration by quantification of centronucleated fibers. Crush muscle injury induced a delay in muscle repair characterized by fibrous tissue in regenerating area. Unstabilized fractures were induced with or without crush muscle injury in muscle surrounding the tibia. Bone repair was assessed by histomorphometric analyses. Muscle injury delayed cartilage formation followed by a severe delay in cartilage removal and bone bridging at later stages of repair leading to pseudoarthrosis. Moreover, muscle injury reduced callus vascularization and increased macrophage recruitment. To assess role of the BMP pathway in bone-muscle interactions, we assessed the effects of bmpr1a inactivation in satellite cells in Pax7CreER;bmpr1afl/fl mice. Our preliminary data at day 7 post-fracture indicate that callus, cartilage and bone formation are not affected in Pax7CreER;bmpr1afl/fl mice compared to controls. When fracture was combined with muscle injury, delayed bone repair was detected as early as day 7 post-fracture. Altogether, these results show that intact muscle is essential for normal bone regeneration and that BMP signaling in satellite cells is required for the regulation of muscle-bone interactions.

Mots-clés

muscle bone musculoskeletal regeneration BMP

PiT1 mediates survival of chondrocytes from endoplasmic reticulum-induced stress in vivo - O2

Greig Couasnay1*, Sarah Beck-Cormier1, Sophie Sourice1, Jérôme Guicheux 1,2, Laurent Beck1.
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Résumé

The chondrocyte is the unique resident cell found in the cartilage and is responsible for synthesis and turnover of the abundant extracellular matrix (ECM). Since the cartilage is avascular, chondrocytes evolve in a harmful microenvironment with low oxygen and nutrient availability. These cellular stresses triggered an evolutionary conserved mechanism known as the unfolded protein response (UPR). Activation of UPR pathway regulates the expression of genes involved in endoplasmic reticulum (ER) homeostasis allowing cell survival and ECM synthesis. If sustained, activation of the UPR causes apoptotic cell death.Our objective is to decipher the cellular functions and physiological role of PiT1, a multifunctional protein expressed in chondrocytes. Our recent findings have demonstrated that PiT1 is a regulator of cell proliferation and apoptosis. Its subcellular localisation in the ER and its ability to promote cell survival led us to study its involvement in the chondrocyte UPR response in vivo. To this aim, we have generated an inducible chondrocyte-specific PiT1 knockout mice (PiT1cKO) model. All pups were injected with tamoxifen at postnatal day 3. Only 48h post tamoxifen injection (p.i), histological analysis of humerus sections revealed the presence of a hypocellular zone in the center of growth plate due to massive cell death, as shown by TUNEL. Analysis of the phenotype onset at earlier stage (8h-24h p.i) showed an upregulation of the ER-associated pro-apoptotic factor, CHOP. Indeed, the ultrastructure of PiT1cKO chondrocytes analysed by TEM displayed a distended ER, a hallmark feature of ER-stressed cells. To explore the implication of PiT1 in this process; we performed in vitro analysis in isolated PiT1cKO chondrocytes and in the ATDC5 chondrogenic cell line. Under ER stress stimuli we demonstrated that PiT1 expression is strongly induced and regulated by three main UPR transducers (ATF6N, ATF4 and XPB1s). Furthermore, PiT1-depleted ATDC5 cells showed an increased sensitivity to ER stress-induced apoptosis as demonstrated by CHOP upregulation. On the contrary, PiT1-overexpressing ATDC5 were more resistant to stress inducers. Our results suggest that under ER stress condition (physiological or pharmacological) PiT1 regulates the mice balance between survival and death in chondrocyte. Of importance the phenotype of PiT1 conditional knockout is reminiscent of HiF1α and PTEN deletion in cartilage. The mechanistic links between HiF1α/ PTEN pathways and PiT1 are currently being investigated in our lab.

Mots-clés

Endoplasmic reticulum stress - chondrocyte - survival

miR-199a/b-5p and Runx2 collaborate to regulate Wnt/β-catenin pathway - O3

Mylène Zarka*1,2, Coline Haxaire1,2 and Valérie Geoffroy1,2
1UMR_S1132 BIOSCAR, Hôpital Lariboisière, Paris; 2Université Paris Diderot

Résumé

Osteoblast differentiation is tightly regulated by cell-cell interactions as well as environmental factors that induce cellular signalling pathway and transcription of specific genes in a temporal and specific pattern. MicroRNAs (miRs) have been shown to regulate several target genes that affect different stages of osteogenic differentiation such as cellular commitment, proliferation, or generation of the bone matrix. By miRome analysis, we previously identified miR-199a/b-5p that is encoded by 3 different miR genes, miR-199a1, miR-199a2 (also called DNM3os) and miR-199b, as positive regulators of osteogenesis. We showed that miR-199a/b-5p expression is positively correlated with osteoblast differentiation and that it acts as an inductor of Runx2 protein expression in pre-osteoblastic cells MC3T3-E1 and primary osteoblasts. Our previous data also suggest that miR-199a/b-5p is implicated in the promotion of osteoblast differentiation. It would acts by directing repression of predicted targets that are inhibitors of osteoblast differentiation such as GSK3β and NLK, two inhibitors of the canonical Wnt pathway. Our working hypothesis is that miR-199a/b-5p regulates the Wnt/β-catenin signaling in osteoblasts dependently or independently of the transcription factor Runx2.First, we demonstrated that miR199a/b-5p can directly regulate the canonical Wnt/β-catenin pathway using TopGal primary osteoblasts. In silico analysis indicated two potential sites for miR-199a/b-5p in the 3'UTR of GSK3β. We showed by transient transfection in MC3T3-E1 cells and in primary osteoblasts that the pre-miR-199a2 and anti-miR-199a2 induced respectively a decrease and an increase in GSK3β at the protein level. Moreover, co-transfection in MC3T3-E1 cells of the pre-miR199-a2 with reporter constructs containing the 0.3kb and 4.0kb fragments of the GSK3β 3_R indicates that miR-199a-5p can inhibit GSK3β expression through the more 3' of its two potential sites. On the other hand, we showed that the transcription factor Runx2 regulates the Wnt/β-catenin pathway in a dose-dependent manner. Moreover overexpression of Runx2 induced a down-regulation of the canonical Wnt/β-catenin pathway in part through the activation of GSK3β expression, and a repression of miR-199a/b-5p expression probably through a negative feedback regulation loop. The down-regulation of the canonical Wnt/β-catenin pathway by miR-199a/b-5p and Runx2, leads to a modification of the RANKL/OPG ratio that may directly impact on osteoclast differentiation and bone resorption. Altogether, our results demonstrated that miR-199-a-5p is able to regulate Wnt/β-catenin signaling directly by inhibition of GSK3β and indirectly by regulating Runx2 expression to control osteoblast differentiation and beyond. Further experiments are still needed in order to decipher the cross regulation between miR-199a/b-5p and Runx2 in osteoblasts.

Mots-clés

osteoblast, microRNAs, Wnt pathway, Runx2

IIB OR NOT IIB: DEVELOPMENT OF AN ORIGINAL SCREENING METHOD BY FLOW CYTOMETRY TO CHARACTERIZE DIVERSE SOURCES OF HUMAN MESENCHYMAL STEM CELLS AND EVALUATE THEIR POTENTIAL FOR CARTILAGE RECONSTRUCTION - O5

Hugo Fabre*1, Maxime Ducret1, 2, 3, Olivier Degoul4, Jonathan Rodriguez5, Emeline Perrier-Groult1, Gianluigi Atzeni4, Odile Damour5, Colin McGuckin4, Nico Forraz4, Elisabeth Aubert-Foucher1, Frédéric Mallein-Gerin1

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Résumé

Articular cartilage is not vascularized and presents poor intrinsic healing potential. Consequently, traumatic and degenerative lesions of articular cartilage eventually progress to osteoarthritis, a worldwide leading source of disability. Joint replacement is a short-term therapy since knee prostheses have limited lifespan and common surgical treatments often lead to the production of fibrocartilage which does not provide correct biomechanical properties to the joint. In this context, interest in cell therapy and tissue engineering for cartilage repair is increasing and currently, autologous chondrocytes represent the most common source of cells that are transplanted although they imply tissue morbidity at the donor site. The use of mesenchymal stem cells (MSCs) represents a promising alternative cellular model but satisfactory protocols allowing proper chondrogenic conversion together with sufficient cartilage matrix production are still missing. Moreover, it is difficult to rank the origin of the MSCs in regard of their potential of proliferation and differentiation from the studies published in the literature since the cell culture conditions used by the research groups are very often different. A main project of our laboratory is to identify the best source of MSCs for cartilage repair.In this study, we undertook an original, polychromatic, 27-marker flow cytometry analysis of MSCs isolated from Wharton's jelly (WJ), adipose tissue (AT), dental pulp (DP), and bone marrow (BM), the latter being the gold-standard for MSCs1. The different categories of MSCs were isolated, expanded and induced towards the chondrogenic lineage in serum-free conditions, with a view of clinical application. Moreover, their chondrogenic conversion was tested not only in micromass (rather a protocol used in basic research), but also in hydrogels, to create tissue-engineered cartilage. Recently, we developed and characterized the first antibody capable of detecting the IIB isoform of human type II procollagen, the only isoform of this collagen type that is expressed by well-differentiated chondrocytes2. Here, we show for the first time that this antibody can be used in flow cytometry to reveal intracellular IIB procollagen expression in MSCs undergoing chondrocyte differentiation in hydrogel. Therefore, this analysis can serve to quantitatively assess the degree of chondrogenic conversion of the MSCs in a biomaterial suitable for cartilage engineering. By using this method, our present efforts aim to compare the chondrogenic potentials of the BM-, AT-, DP- and WJ-MSCs. The results of this study will help to select the most appropriate source of MSCs capable of producing high quality cartilage matrix for cartilage engineering.

Mots-clés

cartilage, chondrocyte differentiation, mesenchymal stem cells, tissue engineering, type II collagen, flow cytometry

Chimie douce, nanostructures minérales et biologie

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Résumé

Lorsque leur concentration dans une solution aqueuse dépasse un certain seuil, les composés minéraux précipitent pour donner des cristaux dont la structure et la morphologie dépendent des conditions expérimentales. Les processus de germination et croissance qui contrôlent la formation de la phase solide peuvent être profondément modifiés en présence de molécules organiques ou biologiques. On obtient alors des mésocristaux hybrides dans lesquels les composantes minérales et biologiques sont étroitement imbriquées.

Depuis des centaines de millions d'années, le vivant a appris à maitriser ces processus biochimiques pour optimiser les propriétés des biomatériaux qu'il élabore. Plutôt que de jouer sur la composition chimique de ces minéraux, il adapte leur nanostructure aux propriétés recherchées. L'exemple des diatomées montre comment avec un même matériau, le verre de silice, ces micro-algues élaborent une coque qui leur permet de jouer avec la lumière pour optimiser leurs propriétés photosynthétiques. L'analyse de ces processus a conduit au dévelopement d'une 'chimie douce' dans laquelle le minéral devient compatible avec la vie.

THE EUROPEAN ABALONE (HALIOTIS TUBERCULATA) SHELL: A RELEVANT MODEL TO STUDY THE IMPACT OF OCEAN ACIDIFICATION - O5

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Résumé

A major environmental concern arises over the acidification and warming of the world's ocean. By the end of the 21st century, surface ocean pH should decrease by 0.1 to 0.3 units, leading to subsequent changes in sea water carbonate chemistry and a reduction in the degree of saturation with respect to calcium carbonate [1]. Due to their need for calcium carbonate ($CaCO_3$) to produce their shell, molluscs are among the most vulnerable invertebrates to acidification with a pronounced sensitivity of larvae and juveniles [2]. Most of the studies to date have focused on the effects of ocean acidification on adult organisms, and more information is needed on the impacts of decreased pH on the early life history stages in order to understand how ocean acidification will affect species distribution and fitness.The present work aimed at evaluating the effects of ocean acidification on the early development and shell calcification of the European abalone Haliotis tuberculata (Mollusca, Gastropododa), an ecologically and commercially important species. Larval development and shell formation have been extensively studied in H. tuberculata, showing that the primary shell is mostly composed of amorphous $CaCO_3$, followed by a gradually crystallization under aragonite [3]. Since aragonite is more susceptible to dissolution compared to calcite, the abalone shell provides a relevant model to study the impacts of ocean acidification.Larval and juvenile abalones, obtained from controlled fertilization held at the hatchery France-Haliotis, were submitted to a range of decreased pH (8.1 to 7.6). The responses of larvae and juveniles were evaluated by measuring the survival rate, morphology and size, growth index and shell calcification. Optical and SEM microscopy were used to assess whether lowering the pH had an influence on shell morphology and microstructure. Our results evidenced that sea water acidification negatively impacted abalone development and disrupted the biomineralization processes, resulting in a delayed shell development. Further studies are in progress to investigate the synergic effects of ocean acidification and warming in order characterize the adaptive responses of the abalone to changing ocean conditions.

Mots-clés

Haliotis tuberculata, Biomineralization, Development , Ocean acidification.

MINERALIZATION OF THE MOUSE TRACHEA IS A SUDDEN AND EARLY PHYSIOLOGICAL EVENT - O6

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Résumé

The trachea is a very complex structure of the respiratory tract, composed of C-shaped cartilaginous rings, made of hyaline cartilage. In contrast to other intensely studied cartilages such as the ones found in the developing growth plate or in the adult joints, very little information is available on the innate propensity of this structure to mineralize. Clinical studies have reported that tracheal calcification is considered a common finding in elderly patients. It is also observed in younger indivduals suffering from rare pathological conditions where the expression or activity of the Matrix Gla Protein (MGP) is impaired, suggesting an important role of this potent Vitamin K-dependent calcification inhibitor in the protection against aberrant mineralization of the trachea. In that context, we ought to understand the cellular and molecular mechanisms at the origin of tracheal mineralization that has been unexplored so far and the potential role played by the BMP and calcification inhibitor MGP in this process. In this study, we thus carried out a thorough anatomical and histological analysis in order to determine the spatiotemporal onset and progression of trachea mineralization during postnatal and adult development of wild-type mice of different strains and in Mgp-deficient mice. This extensive and straightforward analysis led to very unanticipated results. Indeed, in contrast to what has been described in humans, our data undeniably demonstrate that, in the mouse, tracheal mineralization initiates in the cartilaginous rings of wild-type mice as early as one month after birth and progresses through a rostrocaudal direction throughout the trachea to eventually spread in the bronchi after only 2 months. This process seems to be accompanied by the terminal differenciation of the tracheal chondrocytes, as we observed by qPCR and in situ hybridization a strong Collagen X expression preceeding the dynamic mineralization of the cartilage rings. Similar studies performed with Mgp+/+, Mgp+/- and Mgp-/- littermates revealed that a decrease in MGP production in the tracheal environment accelerates the mineralization process of the rings.The present study is the first to describe mouse tracheal calcification and provide evidence that in the mouse, contrary to the typical notion, tracheal cartilage is not a permanent hyaline cartilage throughout life, as it is physiologically able to mineralize early in life possibly through a BMP-dependent mechanism.

Mots-clés

Trachea, mineralization, matrix gla protein, chondrocyte

FGF23 REGULATES MMP13 THROUGH FGFR1 IN HUMAN OA CHONDROCYTES VIA PI-3K AND ERK PATHWAYS - O7

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Résumé

Osteoarthritis (OA) is the most common form of chronic joint disease, characterized by cartilage degeneration that results from complex changes in the chondrocyte phenotype. The presence of phosphate-containing microcrystals in the injured cartilage areas suggests the contribution of the phosphocalcic metabolism in the hypertrophic phenotype-like appearance of some chondrocytes during the disease. As Fibroblast Growth Factor 23 (FGF23) plays a major role in regulating concentrations of inorganic phosphate (Pi), a major inducer of mineralization, FGF23 is an attractive candidate to participate in the phenotype switch of the articular chondrocyte observed in OA. To address this hypothesis, we first compared the expression of FGF23, its receptors (FGFR) and co-receptor (Klotho) in cartilage samples obtained from healthy or OA individuals, then studied the consequences of in vitro FGF23 exposure on chondrocyte phenotype biomarkers and signaling pathways. Immunohistochemistry and gene expression studies were performed on human cartilage samples. Phenotypic biomarkers were studied by quantitative RT-PCR in human OA chondrocytes stimulated with up to 100 ng/ml of FGF23. Collagenase 3 activity was measured by a fluorescent assay. MAPK signaling was investigated by phosphoprotein array, immunoblotting and the use of selective inhibitors. RNA silencing was performed to confirm the respective contribution of FGFR1 and Klotho. In the present study, we showed that FGF23 and FGFR1 expression was 2-fold and 1.4-fold higher in OA chondrocytes than in normal ones, respectively. These overexpressions were confirmed by immunohistochemical analysis of OA cartilage specimens. In addition, Klotho mRNA level was higher in OA chondrocytes, while no significant changes were noticed for FGFR2, 3 and 4. FGF23, FGFR1 and Klotho expression was higher in the damaged regions of OA cartilage. When stimulated with inducing concentrations of FGF23, human OA chondrocytes displayed a sustained expression of FGF23 and markers of hypertrophy such as COL10A1, VEGF and MMP13. Collagenase 3 activity was also increased. At that time, SOX9 expression was down-regulated by FGF23 while that of RUNX2 remained unchanged. We demonstrated further, that FGF23 expression was both FGFR1- and Klotho-dependent whereas MMP13 expression was mainly dependent on FGFR1 alone. Finally, we showed that FGF23-induced MMP13 expression was strongly regulated by the MEK/ERK cascade and to a lesser extent by the PI-3K/AKT pathway. Altogether, the results obtained in this study demonstrate that FGF23 promotes differentiation of OA chondrocytes towards a hypertrophic phenotype and may therefore be considered as an aggravating factor for OA.

Mots-clés

Chondrocytes, FGF23, Osteoarthritis

IN VIVO MEASUREMENT OF PH AT THE SITES OF CALCIFICATION OF THE RED CORAL CORALLIUM RUBRUM - O8

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Résumé

The Mediterranean coral, Corallium rubrum, is famous for its red axial skeleton which has been used for many centuries in jewelry and art ornaments. In addition to this axial skeleton, C. rubrum also produces small micrometer scale biominerals, the sclerites which are distributed amongst the tissues. A calcifying epithelium is responsible for the extracellular formation of the axial skeleton whereas the scleroblasts are responsible for the initial, intracellular steps of sclerites formation. Kinetic experiments with the use of radioactive molecules and pharmacological tools have allowed us to obtain data on the transport of ions from the external seawater to the sites of calcification, suggesting that the process in both cases is active and involves different ion carriers. However no data have been obtained at the cellular level. In the present study we aimed at developing a technique to determine at the cellular level the ionic environment in which precipitation of calcium carbonate (calcite) occurs. We especially focused on one key parameter involved in coral biomineralization, the pH, since the precipitation of calcium carbonate is favored when the pH is increased relative to seawater. We thus developed two preparations: one to measure intracellular pH in isolated scleroblasts and one to measure extracellular pH between the calcifying epithelium and the axial skeleton. We used a pH-sensitive fluorescent probe, carboxyseminaphthorhodafluor-1 (SNARF-1), coupled with inverted confocal microscopy to map C. rubrum pH in the two preparations. This technique allowed us to obtain the first pH measurements in octocoral cells and paves the way for future experiments to look 1) at the mechanistic of pH regulation by ion carriers during the calcification process and 2) at the effect of environmental stressors such as ocean acidification which affects biomineral formation. Indeed it has been shown that the response of coral calcification to acidification depends on the coral's ability to up-regulate pH at the site of calcification, but this response is species-specific and needs to be characterized in C. rubrum.

Mots-clés

Biomineralization - pH - Calcification- Corals

Long chain polyunsaturated fatty acids and bone health.

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Résumé

Over the past years a significant body of research has been published supporting evidence that dietary long chain polyunsaturated fatty acids (LCPUFAs) with a chain length longer than 18C, are beneficial for bone health. Published reviews suggest that LCPUFAs of the n-3 series may prove beneficial when consumed in appropriate amounts. In addition, it has been shown that a reduction of the n-6/n-3 PUFA ratio in the diet and accordingly in membranes of the body could affect bone positively and result in increased bone mass and strength in animals and in humans. Several epidemiological studies have demonstrated a relationship between fat intake and bone health but epidemiological data regarding the intake of LCPUFA from the diet and the relations to bone density of the hip and spine as well as to fracture risk is not conclusive. A large number of animal studies have shown that LCPUFAs affect bone mineral content and bone mass, thereby affecting bone formation in growing animals. In the ovariectomised female rat, LCPUFAs, especially docosahexaenoic acid (DHA) reduced bone loss arising due to lack of oestrogen. Several of the LCPUFAs have been shown to affect bone cells, i.e. osteoclasts and osteoblasts, via varying cellular signaling pathways or growth factors, thereby affecting bone formation and resorption. LCPUFAs have been shown to affect cellular proteins and receptor activator of nuclear factor κβ (RANK), reduce the synthesis of prostaglandin E2, suppress inflammatory cytokines and give rise to lipid mediators which are anti-inflammatory and thereby reduce bone resorption. Our most recent work in murine RAW264.7 cells and human CD14+ monocytes indicated that DHA decreases osteoclastogenesis, resorptive capacity of the osteoclasts and downregulates the expression of several genes including c-Fos, NFATc1, RANK and DC-STAMP. LCPUFAs therefore play an important role in regulating osteoclast activity and are bone protective.

Mots-clés

Long chain fatty acids; bone density; osteoclasts; osteoblasts; cytokines

Effects of Osteum, a natural ingredient, containing micellar calcium, vitamin D and K2, on bone mineral density - O9

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Résumé

The aim of postmenopausal osteoporosis treatment is to decrease bone resorption and/or increase bone formation. Because of the slow bone turnover, osteoporosis prevention and therapies are long-lasting, implying great costs and poor compliance. Even if the effect of nutrition on bone is not as marked as that of pharmaceutical agents, it can be of great help. The nutritional intervention can be done alone preventively or as an adjuvant therapy in more severe cases. Some nutritional components such as calcium and vitamin D are recognized to have a positive effect on bone. The purpose of our study was to demonstrate the efficiency of OsteumTM, a natural ingredient including micellar calcium, vitamin D and K2, to improve bone mineral density (BMD).The in vitro study, using primary murine bone cells, showed that OsteumTM does not modulate cell growth but that it is able to stimulate osteoblast differentiation as shown by increase ALP activity and mineralization and to inhibit osteoclast's differentiation and resorption activity as shown by reduction of bone resorption. In vivo, using a model of ovariectomized mice, we showed that BMD was dose-dependently improved after OsteumTM ingestion. We also report increased osteoblast activity as shown by increase of ALP activity and decreased osteoclastogenesis as shown by reduced CTX activity. Improvement in BMD was observed after an intervention period of at least 8 weeks. This suggests that the acute effect of OsteumTM is mild but, when chronically ingested, the effect was found to be significant. Our results show that a dairy product providing not only calcium and vitamin D but also vitamin K is more efficient than calcium and vitamin D alone for BMD improvement.

Mots-clés

Osteoporosis, Bone mineral density, cell culture, ovariectomized mice model

Osteoclast activity regulates hematopoietic stem cell niches during inflammatory bowel disease - O10

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Résumé

Inflammatory bowel disease (IBD) is a chronic disease characterized by a severe inflammation of the gastrointestinal tract. Bone loss has been reported in more than 40% of patients with IBD and remains a major extraintestinal cause of morbidity. We have recently demonstrated that the bone marrow (BM) Th17 TNFa cells induce osteoclastogenesis in a murine model of IBD. Indeed, this study revealed that these T cells are able to recruit osteoclast (OCL) progenitor cells to the BM and link chronic inflammation to bone destruction. Hematopoietic stem cells (HSCs) mostly reside in the BM, where they undergo proliferation and multi-lineage differentiation, giving rise to mature leukocytes (lymphoid and myeloid progenitors), which are released in turn to the blood in order to carry out their function. In IBD patients, high numbers of myeloid cells in the inflamed intestine correlates with the clinical disease activity. On the other hand, it has been described that OCLs are able to promote HSCs proliferation and egress from their niches in inflammatory conditions. The aim of the present study was to investigate the effect of osteoclast activity on the egress, mobilization and differentiation of HSCs during IBD. We used the well characterized model of IBD induced by the transfer of naive CD4+ T cells into the Rag1-/- mice which is associated with bone loss. To block the osteoclast activity, we injected zoledronic acid (ZA) into Rag1-/- mice during 6 weeks before the transfer of naive CD4+ T cells. Our results show that blocking osteoclast activity resulted in a decrease of clinical signs of IBD. As expected, we have observed an increase of HSC number associated to their high proliferation rate in the BM of IBD mice. In parallel, we have found an accumulation of myeloid cells in the spleen and the colon. In contrast, in IBD mice treated with ZA, HSCs did not proliferate in the BM. However, hematopoeisis increases in the spleen. In conclusion, these results suggest that the increased activity of OCLs observed in IBD participate to mobilization of HSCs and the exacerbation of the disease.

Mots-clés

Osteoimmunology, hematopoietic niches, osteoclasts.

Deficiency in claudin-16 tight junction protein as novel cause of Amelogenesis Imperfecta - O11

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Résumé

Mutations in the CLDN16 gene encoding claudin-16, a tight junction (TJ) membrane-bound protein, lead to Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis (FHHNC). Oral examination was performed in five unrelated patients with CLDN16 mutations. The expression and potential role of CLDN16 were investigated in the forming tooth germ, using the murine model of FHHNC. Amelogenesis Imperfecta (AI) was diagnosed in all the patients and the Cldn16 knockout (KO) mice presented similar phenotype with cusp fracture in molars. The expression of claudin-16, described primarily in the kidney, was demonstrated here in the TJ of murine tooth germs. It was located at the distal end of secretory ameloblasts and its absence strongly modified TJ organization. The forming enamel matrix in Cldn16 KO mice displayed a significantly lower pH value when compared with WT, associated with decreased matrix metalloproteinase (Mmp) activity, and accumulation of enamel matrix proteins.This study highlights an association of FHHNC due to CLDN16 mutations with Amelogenesis imperfecta (AI) and underlines the need to establish a specific dental follow-up for these patients.

Mots-clés

tight junction, secretory ameloblast, Mmp, pH, enamel matrix protein

The polyphenol fisetin protects bone by repressing NF-kB and MKP-1-dependent signaling pathways in osteoclasts - O12

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Résumé

Osteoporosis is a bone pathology leading to increase fractures risk and challenging quality of life. Since current treatments could exhibit deleterious side effects, the use of food compounds derived from plants represents a promising innovative alternative due to their potential therapeutic and preventive activities against human diseases. In this study, we investigated the ability of the polyphenol fisetin to counter osteoporosis and analyzed the cellular and molecular mechanisms involved. In vivo, fisetin consumption significantly prevented bone loss in estrogen deficiency and inflammation mice osteoporosis models. Indeed, bone mineral density, micro-architecture parameters and bone markers were positively modulated by fisetin. Consistent with in vivo results, we showed that fisetin represses RANKL-induced osteoclast differentiation and activity as demonstrated by an inhibition of multinucleated cells formation, TRAP activity and differentiation genes expression. The signaling pathways NF-kB, p38 MAPK, JNK and the key transcription factors c-Fos and NFATc1 expressions induced by RANKL, were negatively regulated by fisetin. We further showed that fisetin inhibits the constitutive proteasomal degradation of MKP-1, the phosphatase that deactivates p38 and JNK. Consistently, using shRNA stable cell lines, we demonstrated that impairment of MKP-1 decreases fisetin potency. Taken together, these results strongly support that fisetin should be further considered as a bone protective agent.

Mots-clés

Bone, polyphenol, osteoclast, NF-kB, MKP-1

HIF signaling in skeletal progenitors promotes breast cancer growth and metastasis through systemic production of CXCL12 - O13

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Résumé

High bone mineral density (BMD) has long been associated with increased risk of breast cancer. Conversely, low bone mass has been correlated with lower risk of breast cancer. Although BMD was initially thought to reflect a cumulative exposure to estrogens, recent clinical trials demonstrated that high bone mass correlates with elevated breast cancer incidence independently of reproductive correlates, endogenous and exogenous exposure to estrogen. However, the biological mechanism linking bone mass and the risk of breast cancer is unknown. Here we show that osteoprogenitor cells, targeted by Osterix driven Cre-recombinase, exert a systemic control of breast cancer growth and metastasis. Deletion of the tumor suppressor gene von Hippel Lindau (Vhlh) specifically in mouse osteoprogenitors (Osx/Vhlhfl/fl), which results in increased protein level of the Hypoxia-Inducible Factor-1alpha (Hif-1alpha) in these cells, led to increased bone mass, and increased mammary tumor growth and metastasis. Conversely, deletion of Hif-1alpha in osteoprogenitors (Osx/Hif-1alphafl/fl) decreased bone mass, and dampened mammary tumor growth and metastasis. We found that changes in the bone microenvironment are associated with changes in the plasmatic levels of the chemokine C-X-C motif ligand 12 (CXCL12). Pharmacological inhibition of the CXCL12-CXCR4 pathway abolished increased primary tumor growth and dissemination in Osx/Vhlhfl/fl mice. Therefore, skeletal dysfunction alters tumorigenesis beyond the bone microenvironment. Our results provide a mechanistic explanation as for why high bone mass is linked to increased risk of breast cancer, and support the notion that the skeleton is an important organ of the tumor macroenvironment. They also indicate that drugs affecting bone homeostasis may have important consequences in breast cancer.

Mots-clés

Bone metastasis, Bone microenvironment, Breast cancer, Osteoblast, Hypoxia

OSTEOCLASTS EXPRESSING CX3CR1 INDUCE TNFα-PRODUCING CD4+ T CELLS IN INFLAMMATORY CONDITION - O14

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Résumé

Chronic inflammatory diseases, such as inflammatory bowel disease (IBD), are characterized by a severe bone destruction mediated by an increased osteoclast (OCL) activity. OCLs are multinucleated cells derived from monocytes (MN-OCLs) in steady state, but also from dendritic cells (DC-OCLs) under inflammatory conditions. In addition to their bone resorptive function, OCLs also have an immunomodulatory function. Indeed, recent studies revealed that OCLs are antigen-presenting cells. In this context, we have observed that in vitro differentiated MN-OCLs and DC-OCLs have opposite immunomodulatory functions: MN-OCLs induce CD4+ regulatory T cells whereas DC-OCLs induce inflammatory CD4+ T cells in an antigen-specific manner. However, up to now, no specific markers are available to identify tolerogenic and inflammatory osteoclasts and to study their immunomodulatory effect in vivo.Our aim was to identify such surface markers and to characterize the corresponding OCL subsets in normal and in inflammatory conditions.We have analyzed about 20 surface markers described for monocytes and dendritic cells and we have observed an increased proportion of cells expressing CD39 and CX3CR1 in inflammatory DC-OCLs compare to MN-OCLs. We used the well characterized model of IBD induced by the injection of naive CD4+ T cells into Rag1-/- mice, which associates IBD and bone destruction, to analyze OCLs derived from the bone marrow (BM) of IBD and control mice. OCLs from IBD mice present inflammatory properties similar to DC-OCLs. They induce CD4+ T cell proliferation and the differentiation of CD4+ T cells producing TNFα and IFNγ. In contrast, OCLs from healthy control mice have no effect on CD4+ T cell proliferation and induce CD4+ FoxP3+ regulatory T cells, as do MN-OCLs. Interestingly, compared to the control mice, the proportion of CX3CR1+ OCLs is strongly increased in the IBD mice but not the proportion of CD39+ OCLs. We have confirmed the increase of CX3CR1+ OCLs after the in vivo induction of sever bone destruction by RANK-L injection compared to untreated mice. Lastly, we have also showed that CX3CR1+ OCLs induce inflammatory CD4+ T cells but not regulatory T cells.Our results demonstrate that OCLs in steady state are related with the BM tolerance, probably avoiding self-reactivity against the peptides continuously produced during bone resorption. In contrast, in conditions of IBD or increased RANK-L level, OCLs acquire an inflammatory phenotype characterized by CX3CR1 expression probably participating in inflammatory or autoimmune responses. The implication of CX3CR1 in the immunomodulatory function of OCLs is under investigation to determine if it could represent a new therapeutic target to control inflammatory bone destruction.

Mots-clés

osteoimmunology, osteoclast, inflammation

CALPAIN-6 EXPRESSION IDENTIFIES A STEM CELL POPULATION IN OSTEOSARCOMA - O15

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Résumé

Identification of cancer stem cells in carcinomas has proved to be useful to understand cancer progression and for prognostic purpose. However, the properties of osteosarcoma stem cells remain challenging and controversial, mainly due to the lack of functional markers to study such cells in vivo. Previously, we identified calpain-6 as a protective factor involved in chemoresistance process of osteosarcoma. To investigate the mechanisms controlling its expression we characterized 7285 bases of the regulatory sequence in calpain-6 gene. This sequence comprises an active promoter and multiple functional binding sites for embryonic stem cell factors such as Oct4, Nanog and Sox2 as shown by Rapid cDNA End amplification and Chromatin precipitation. Silencing Oct4, Nanog or Sox2 was sufficient to reduce basal and hypoxia dependent up-regulation of calpain-6 expression and the activity of the regulatory sequence cloned upstream the luciferase gene reporter. This indicates that calpain-6 is controlled by the stem cell transcription factors. To further document a possible relationship between Calpain-6 and a stem cell phenotype, we used GFP as gene reporter, to identify the cells in which the calpain-6 promoter was activated. Culturing osteosarcoma cell lines on non-adherent plastic and in minimal medium allowed obtaining spheroids that were previously shown to be enriched in tumorigenic stem-like cells. Calpain-6 protein was up regulated in spheres obtained from human 143B cells as compared to adherent cultures. Moreover, GFP positive cells sorted from adherent cultures have higher capacities to form spheroids than GFP negative cells. These GFP positive cells also expressed higher RNA levels of the embryonic stem cell markers, c-MYC and ABCB1. Five weeks after injection into the tibia of BALB/c mice, GFP-positive K7M2 cells formed tumours that produced a high luminescent signal as compared to tumours formed from GFP-negative cells that are largely necrotic. In in vitro scratch tests, migrating cells were found to express high levels of calpain-6 and GFP-positive cells displayed higher capacities for migration than negative ones, whereas, calpain-6 shRNA reduced these capacities. Finally, intra bone injection of GFP-positive cells resulted in more metastatic lesions in lungs than negative cells indicating that calpain-6 is involved in metastatic process.Altogether our data show that calpain-6 expression is regulated by transcription factors that control multipotency and renewal of embryonic stem cells. Calpain-6 identifies an osteosarcoma cell population that express stem markers and with higher chemoresistance, migration capacities and tumorigenicity. The reporter system driven by calpain-6 regulatory sequence may therefore represent a powerful tool to further study stem cells in osteosarcoma.

Mots-clés

stem cell, osteosarcoma, calpain-6

miR-146a deficiency in Ly6Chigh monocytes contributes to pathogenic bone loss during inflammatory arthritis - O16

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Résumé

Background and objectives: Increased activity of bone-resorbing osteoclasts (OC) activity contributes to bone resorption during osteoporosis and rheumatoid arthritis (RA). Negative regulation of osteoclastogenesis is thus critical to restore bone homeostasis and to prevent pathological bone erosion. Among regulatory molecules, microRNAs play important roles under physiological and pathological conditions and represent innovative therapeutic targets, but remain poorly studied in the context of osteoclastogenesis. Considering that monocytes exist in two main subsets committed to different functions, and that the classical Ly6Chigh monocyte subset represents main OC precursors (OCP), we investigated whether miRNAs differentially expressed between monocyte subsets regulate osteoclastogenesis in vivo and could represent potential target to intefere with bone loss in RA. Materials and Methods: Genome-wide miRNA expression study identified miRNAs differentially expressed between classical and non-classical monocyte subsets sorted from mouse (Ly6Chigh and LyC6low) and human (CD14+CD16- and CD14dimCD16+) blood. OCP sorted from miR-146a deficient mice or control littermates were used for in vitro differentiation assays. Two mouse models of pathological bone erosion were used: K/BxN serum transfer arthritis and ovariectomy. Disease severity was assessed clinically and histologically, and in vivo bone histophotometry analyzed. Intravenous injection of miR-146a mimic-containing lipoplexes was used to rescue miR-146a expression on Ly6Chigh monocyte in the mouse model of collagen-induced arthritis. Disease severity, inflammation, bone erosion and efficiency of miRNA mimics delivery to cell subset were monitored. Results: We identified miR-146a as the most differentially expressed miRNA between classical and non-classical monocytes. MiR-146a was down-regulated during OC differentiation and in OCP isolated from arthritic individuals as compared to healthy controls. Knockdown of miR-146a in OCP increased osteoclast differentiation in vitro. While no bone phenotype was evidenced in miR-146a deficient mice, neither under steady state nor under ovariectomized-induced osteoporosis conditions, arthritis-induced bone erosion was increased in miR-146a knockout mice. Finally, delivery of miR-146a mimics to Ly6Chigh monocytes efficiently interferes with pathological bone loss in inflammatory arthritis, reducing OC counts and erosion in inflamed joints as well as ex vivo osteoclastogenic differentiation capacity of OCP. This clinical benefit was associated an upregulation of the miR-146a/RelB axis in Ly6Chigh monocytes. Conclusions: Overall, the present work suggests that miR-146a is a sensor that negatively regulates osteoclastogenesis in OCP during inflammatory arthritis. We showed that reduced expression of miR-146a in Ly6Chigh monocytes is responsible for increased osteoclastogenesis in inflammatory arthritis and that delivery of miR-146a mimics to Ly6Chigh monocytes may offer valuable therapeutic strategy to interfere with pathological bone loss.

Mots-clés : miR-146a, monocyte subsets, osteoclast, arthritis

Macrophages and BM stem cell niches: the roles of PGE2 and coagulation

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Résumé

Blood forming, hematopoietic stem cells (HSC) functionally express the chemokine receptor CXCR4, which is required to control their directional motility, cell cycle, adhesion and bone marrow (BM) repopulation. HSC are mostly BM retained in a quiescent, non-motile mode via CXCR4 induced adhesion interactions with BM niche cells, which functionally express surface CXCL12. Surface CXCL12 mediated adhesion interactions with BM stromal cells protect quiescent, CXCR4+ stem cells from DNA damaging agents, while preserving their developmental potential. On the other hand, CXCL12 secretion by BM stromal cells and its release to the blood increase CXCR4 expression and signaling in hematopoietic stem and progenitor cells (HSPC), inducing their egress and clinical mobilization. Finally, CXCR4+ HSC also follow CXCL12 gradients to the BM when they home back from the blood. Intriguingly, dynamic CXCR4/CXCL12 signaling cascades control not only HSC retention in the BM, but also their homing to the BM and their release and mobilization to the blood.

In addition to their central role in host defense, myeloid cells participate in organ homeostasis, including HSC localization. Monocyte-derived bone resorbing osteoclasts cleave and release endosteal membrane-bound CXCL12, SCF and osteopontin, factors needed for HSC adhesion and BM retention, leading to CXCL12/CXCR4 mediated HSPC mobilization. Recently, we identified a rare population of BM αSMA+ macrophages that highly express COX-2, which protects HSC from inflammatory insults via COX-2-mediated PGE2 secretion and ROS inhibition. In vitro PGE2 upregulates CXCR4 on enriched human cord blood CD34+ and murine HSC via cAMP activation, leading to enhanced CXCL12 induced migration, homing and BM repopulation. However, preliminary results reveal that in vivo PGE2 treatment reduced CXCR4 expression on BM HSC by increasing surface CXCL12 expression by stromal cells. Surface CXCL12 upregulation is due to PGE2 mediated secretion and autocrine signaling of lactate, which is followed by cAMP inhibition in BM stromal cells. Indeed, antagonizing COX-2 or activating cAMP induced HSPC mobilization, via BM CXCL12 secretion and increased CXCR4 expression and signaling in HSPC.

Coagulation cascades also navigate HSC localization. We revealed that anticoagulant microenvironments in the BM mediate HSC adhesion and retention via inhibition of nitric oxide (NO) production and HSC migration. Physiologic stress induced extensive production of the pro-coagulant factor thrombin, which activates NO generation, CXCL12 secretion and enhanced CXCR4+ stem cell motility and mobilization. We found multinucleated pre-osteoclast cell clusters in femoral metaphysis, expressing Tissue Factor (TF) a potent initiator of coagulation leading to thrombin generation. Osteoclast maturation and stress signals also activate TF induced pro-coagulation cascades, mediating HSPC egress and recruitment to the circulation.

Daily light and darkness cues regulate many physiological processes, including osteoclast/osteoblast bone remodeling, BM CXCL12 production and secretion and CXCR4+ HSC egress. In addition to the previously identified morning peak of HSC egress to the blood, we identified two peaks of BM HSC proliferation: a morning peak in conjunction with stem cell egress, and an evening peak of HSC expansion without egress. Higher BM levels of the HSC-protecting aSMA+ monocyte/macrophages documented in the evening and their associate “low-CXCR4/high CXCL12” BM microenvironment provide the mechanism for these fluctuating patterns. Finally, we found higher PGD2 levels and reduced PGE2 levels in monocyte/macrophages in the morning, whereas at night we documented the opposite patterns. PGD2 induced CXCL12 secretion and HSC egress, while PGE2 induced their retention.

Our studies attribute central roles for BM myeloid cells in HSC regulation.

Réponses adaptatives du squelette à la contrainte mécanique : rôle de la périostine

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Résumé

Over the past years a significant body of research has been published supporting evidence that dietary long chain polyunsaturated fatty acids (LCPUFAs) with a chain length longer than 18C, are beneficial for bone health. Published reviews suggest that LCPUFAs of the n-3 series may prove beneficial when consumed in appropriate amounts. In addition, it has been shown that a reduction of the n-6/n-3 PUFA ratio in the diet and accordingly in membranes of the body could affect bone positively and result in increased bone mass and strength in animals and in humans. Several epidemiological studies have demonstrated a relationship between fat intake and bone health but epidemiological data regarding the intake of LCPUFA from the diet and the relations to bone density of the hip and spine as well as to fracture risk is not conclusive. A large number of animal studies have shown that LCPUFAs affect bone mineral content and bone mass, thereby affecting bone formation in growing animals. In the ovariectomised female rat, LCPUFAs, especially docosahexaenoic acid (DHA) reduced bone loss arising due to lack of oestrogen. Several of the LCPUFAs have been shown to affect bone cells, i.e. osteoclasts and osteoblasts, via varying cellular signaling pathways or growth factors, thereby affecting bone formation and resorption. LCPUFAs have been shown to affect cellular proteins and receptor activator of nuclear factor κβ (RANK), reduce the synthesis of prostaglandin E2, suppress inflammatory cytokines and give rise to lipid mediators which are anti-inflammatory and thereby reduce bone resorption. Our most recent work in murine RAW264.7 cells and human CD14+ monocytes indicated that DHA decreases osteoclastogenesis, resorptive capacity of the osteoclasts and downregulates the expression of several genes including c-Fos, NFATc1, RANK and DC-STAMP. LCPUFAs therefore play an important role in regulating osteoclast activity and are bone protective.

Mots-clés

Long chain fatty acids; bone density; osteoclasts; osteoblasts; cytokines

Rôle fonctionnel du récepteur à l'acide lysophosphatidique (LPA1) au cours de la différentiation ostéoclastique et de la résorption osseuse - O17

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Résumé

Le LPA (acide lysophosphatidique) est un bio phospholipide qui agit comme un facteur de croissance, influençant nombre de processus cellulaires comme la prolifération, la différenciation et la migration (1). Le LPA est produit par l'activité phospholipase D de l'autotaxine sur l'acide phosphatidyl-choline (LPC, lipide très abondant dans le sang circulant). Le LPA agit via six récepteurs membranaires (LPA1-6) associés à différentes classes de protéines G, presque toutes les cellules eucaryotes expriment au moins un de ces récepteurs ce qui rend l'étude de la signalisation par le LPA extrêmement complexe (2). Par le passé, nous avons montré l'implication du récepteur LPA1 au cours de l'ostéoclastogénèse établie à partir des cellules mononuclées de la moelle osseuse de souris (3). Cependant, le récepteur LPA1 est exprimé par toutes les cellules de l'os, et son rôle dans le remodelage osseux n'est pas clairement défini. En effet, bien que les souris LPA1-/- présentent un déficit global de masse osseuse, nous démontrons ici que l'ostéoclastogénèse est réduite dans les souris LPA1-/- alors que ce n'est pas le cas pour les souris LPA2-/- et LPA3-/-, LPA2 et LPA3 étant des récepteurs plus faiblement exprimés dans les ostéoclastes. En accord avec ce résultat, nous avons trouvé que, seule l'expression du récepteur LPA1 était fortement induite au cours de la différentiation ostéoclastique in vitro et que différents inhibiteurs du LPA1 (Ki16425 , Debio0719, VPC12249) bloquent tous l'osteoclastogénèse. Le blocage du récepteur LPA1, par Ki16425 se traduit par l'inhibition de l'expression du facteur de transcription NFATC1 (nuclear factor of activated T-cell cytoplasmic1) et DC-STAMP (dendritic cell specific transmembrane protéine). De ce fait, ce sont les étapes de fusion et les phases tardives de la différentiation qui sont affectées. Nous avons ensuite examiné l'activité de résorption des Ocs (ostéoclastes) LPA1-/-, ils présentent une diminution de résorption de la matrice minéralisée, et comme les Oc WT traités avec l'inhibiteur Ki16425, les structures du cytosquelette d'actine sont altérées. Les Ocs LPA1-/- matures ont un nombre réduit de zone de scellement ou de ceintures périphériques d'actine, lorsqu'ils sont cultivés sur un support minéralisé ou pas, ce qui explique leur diminution d'activité de résorption. De façon remarquable, nous avons observé une très forte élévation du niveau d'expression du transcrit LPA1 dans les os issus de souris ovariectomisées (x11,5 fois le niveau des souris SHAM) , alors que les autres récepteurs au LPA ne varient pas. Ce résultat identifie LPA1 comme une nouvelle cible thérapeutique pour prévenir la perte de masse osseuse. Confirmant cette hypothèse, nous avons pu montrer que dans les souris ovariectomisées mais traitées aux biphosphonates, le niveau de LPA1 n'augmente pas fortement et surtout qu'un traitement systémique des souris par le Debio0719 empêche la perte osseuse induite par ovariectomie. Par ailleurs nous avons pu constater par vidéo timelapse et microscopie biphotonique que cet inhibiteur augmente la migration des précurseurs ostéoclastiques aussi bien in vitro qu'in vivo (4).

Mots-clés

acide Lysophosphatidique, LPA1, osteoclast, différentiation, résorption osseuse

Primary cilia of the cartilage is abnormal in FGFR3-related disorders - O18

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Résumé

Achondroplasia (ACH) and hypochondroplasia (HCH) are the most frequent dwarfism. Mutations in Fibroblast Growth Factor Receptor 3 (FGFR3) gene, encoding for a tyrosine kinase related receptor, have been identified in ACH, HCH and in a lethal dwarfism, thanatophoric dysplasia (TD). FGFR3 mutations lead to the constitutive activation of the receptor, resulting in excessive phosphorylation and disruption of endochondral ossification, growth plate disorganization, chondrocyte proliferation and maturation through various signaling pathways. We hypothesized that this growth plate defect could be due to anomalies of the primary cilia (PC) of the cartilage cells. In agreement, it was previously reported that in mutant dwarf mice with IFT88 mutation there is growth plate anomalies similarly to those observed in FGFR3-related dwarfism. IFT88 protein plays a predominent role in ciliogenesis and it is involved in ciliary transport.In order to confirm our hypothesis, we used multiple immunostainings to analyze PC length in human and mouse ACH and TD chondrocytes with relevant markers. Axonema of PC is characterized by the presence of Arl13b, a small G protein involved in ciliary transport, and tubulin acetylation. We performed immunostainings (Z-stack confocal imaging then volume 3D reconstruction) and revealed basal bodies of PC via gamma-tubulin immunostaining and ciliary axonema. We also evaluate FGFR3 localization, the role of cytoskeleton network (actin), cell cycle abnormal activation (Ki-67), and Ihh pathway activation (smoothened and Gli proteins).Here, we provide evidence that FGFR3 mutation in ACH human and TD mouse chondrocytes significantly induces length reduction of PC. We also found a colocalization between Arl13b and acetylated tubulin that confirm PC reduced-length. We also characterized PC parameters of mouse and human chondrocytes. Currently we are analyzing FGFR3 localization, cytoskeleton network, cell cycle over-activation and both PC localization and straightness.In conclusion, we showed for the first time that ACH and TD belong to “ciliopathy” group. Our study provides new insight into the role of primary cilia in FGFR3-related chondrodysplasias and could offer novel perspective for therapeutic approaches.

Mots-clés

Primary cilla, FGFR3, Achondroplasia, Growth plate

INVESTIGATION OF THE CALCIUM-SENSING RECEPTOR EXPRESSION IN MONOCYTES ISOLATED FROM SYNOVIAL FLUIDS - P1

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Résumé

Introduction: Given the influence of pro-inflammatory cytokines on calcium-sensing receptor (CaSR) expression, we assessed CaSR expression in monocytes isolated from synovial fluid of patients with different types of rheumatisms and explored whether CaSR expression was related to the inflammatory nature of the synovial fluid. Methods: Pilot, cross-sectional, monocentric study in which were included all patients who presented with an articular effusion in the rheumatology ward. Surface and total CaSR expressions in monocytes isolated from synovial fluid and blood were assessed by flow cytometry analysis. U937 cells were cultured during 24 hours in presence of cell-free synovial fluids in order to specify the influence of different synovial fluids on CaSR expression in vitro. Results: Forty one patients were included: osteoarthritis (n=10), microcristallin rheumatisms (n=10), rheumatoid arthritis (n=12) and other inflammatory rheumatisms (n=9). In monocytes isolated from synovial fluid, the measure of CaSR expression (surface and total) shows that local pathological conditions influence CaSR expression. Indeed, a significant decrease of CaSR expression is observed between the osteoarthritis group and the 3 other groups with inflammatory rheumatisms and also between the microcristallin rheumatism group and the rheumatoid arthritis group. Accordingly, CaSR expression in monocytes isolated from peripheral blood of the same patients was shown to be significantly decreased in rheumatoid arthritis patients compared to osteoarthritis. However, in circulating monocytes no other significant difference in CaSR expression were observed between groups, suggesting that CaSR expression in monocytes isolated from synovial fluid could be a marker of interest in rheumatic diseases. Confirming these data, CaSR expression was shown to be increased in a dose dependant manner in vitro when U937 cells were incubated with synovial fluid from osteoarthritis patients. This effect was significantly lowered when “inflammatory” synovial fluids were used. Discussion: As circulating monocytes, monocytes isolated from synovial fluid express CaSR. However, at the individual level, there are some differences in CaSR expression in monocytes according to their environment of isolation. This can be explained in one hand by the influence of synovial fluid on CaSR expression and on the other hand by the recruitment of a specific sub-population of monocytes in the synovial fluid. Further experiments are needed to address these questions. Conclusion: Together, our results indicate that CaSR expression measured in monocytes isolated from synovial fluid is influenced by extracellular microenvironment and suggest that it could be used as a complementary biomarker in some difficult diagnosis to differentiate the pathological conditions responsible for articular effusion.

Mots-clés

calcium-sensing receptor, monocytes, osteoarthritis, rheumatoid arthritis, synovial fluid

HYPOXIA-DEPENDENT DNA METHYLATION TO CONTROL THE CHONDROCYTE PHENOTYPE - P2

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Résumé

Background: Articular cartilage is an avascular tissue that normally displays a low oxygen concentration. The chondrocytes are thus adapted to hypoxic environment, and are the unique resident cells to produce the cartilage matrix components such as type II collagen and aggrecan. Cartilage injury or degenerative processes can lead to large defects of the articular cartilage surface such as observed in osteoarthritis (OA), and eventually cause pain and important joint disabilities. But articular cartilage has a reduced regenerative capacity, and actual therapies are based on the use of chondrocytes and their manipulation in vitro such as Autologous Chondrocyte Transplantation. It implies a monolayer expansion of chondrocytes in vitro. However, in such standard culture conditions, chondrocytes undergo dedifferentiation into fibroblast-like cells, resulting in the formation of a fibrocartilage. Maintaining the differentiated chondrocyte phenotype remains therefore a major challenge to promote the correct cartilage matrix production. We have recently shown with a detailed microarray study, that hypoxia is a crucial signal since it stimulates the chondrocyte phenotype by up-regulating the expression level of key chondrocyte markers, and several cartilage matrix genes (Lafont et al., 2008). On the other hand, accumulating evidences support the role of DNA methylation/demethylation process in the aberrant expression of chondrocyte specific genes in OA (Rushton et al., 2014 and Watson et al., 2010).Aim: In this study, we investigate the role of the DNA methylation of anabolic and catabolic genes in chondrocytes cultured under hypoxic environment. Results: Healthy articular cartilage was obtained from knees of four donors. To correlate differences in the DNA methylation with the gene expression, we used the same experimental conditions as the previous microarray study. We performed the Infinium HumanMethylation450 BeadChip array (an array that covers CpG sites in the human genome including CpG islands, shores, shelves and open sea), and compared the DNA methylation profile of differentiated chondrocytes and dedifferentiated chondrocytes incubated under 20% and 1% oxygen concentrations. To identify new chondrocyte-specific methylation signatures and determine the unwanted fibroblast-like signature, we also compared the DNA methylation profile of primary cultures of human fibroblasts. Conclusion: Our study will determine new DNA methylation mechanisms induced by hypoxia and involved in the regulation of chondrocyte genes.

Mots-clés

Cartilage, Differentiated chondrocytes, Hypoxia, DNA methylation, Epigenetics, Cartilage engineering

A NEW ALTERNATIVE IN BONE REGENERATION: COMBINATION OF CHITOSAN / HYDROXYAPATITE SCAFFOLD, NACRE ACTIVE COMPOUNDS AND STEM CELLS - P3

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Résumé

Current treatments for large bone defects are not completely satisfying due to the lack of vascularisation into the newly formed tissue. The major challenge in bone tissue engineering is the creation of a bone substitute compensating these defects. Our strategy to obtain a biocompatible and vascularised bone substitute is based on two axes. First, we will study the differentiation of two types of cells (mesenchymal stem cells (MSC) and endothelial progenitor cells (EPC)) induced by nacre active compounds. In a second time, we will synthesize a three-dimensional scaffold composed by chitosan and hydroxyapatite nanoparticles (HNP) to allow cell adhesion and differentiation.First, we isolated the cells from human umbilical cord blood or human bone marrow. Then, the cells were treated with nacre active compounds at different concentrations, to enable osteogenic differentiation of MSCs and endothelial differentiation of EPCs. The osteogenic differentiation of MSC into osteoblasts (OBL) is evalued by qPCR (ColX, Osteopontin...) and alizarin red staining. The endothelial differentiation of EPC into endothelial cells (EC) is monitored by flow cytometry, qPCR and western blot (CD31, VEGFR-2...). The functionality of the cells obtained was shown by an in vitro angiogenesis assay. In a second time we used a material extracted from biomass, chitosan. Chitosan is used to produce a hydrogel by chemical or physical crosslinking. HNP are obtained by sol/gel method or coprecipitation to control size, composition and shape. The nanoparticles are dispersed in the chitosan hydrogel. The HNP are characterized chemically and structurally before and after grafting by several techniques (Transmission Electron Microscopy, Dynamic Light Scattering and X-ray Diffraction).The results obtained on both cells types highlight the positive effects of nacre active compounds on osteogenic differentiation of MSC and endothelial differentiation of EPC. The tridimensional scaffold enriched with HNP and coupled with cells and nacre active compounds is a promising strategy to create an original bone tissue with biological properties similar to those of the native one.

Mots-clés

osteogenic differentiation ; angiogenic differentiation ; nacre active compounds ; chitosan ; hydroxyapatite nanoparticles

Hydrolyzed Collagen Contributes To Osteoblast Differentiation In Vitro And Subsequent Bone Health In Vivo - P4

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Résumé

Collagen I is the main component of organic bone matrix. Its correct synthesis, folding and degradation are crucial for bone homeostasis. According to this pivotal role in bone structure, we investigated the potential health benefits of hydrolyzed collagen (HC) from different origin on bone using translational approaches.

Regarding the influence of HC on bone forming cells in vitro, we first insured the absence of cytotoxicity of HC addition in culture media. Consistently, as compared to BSA control conditions, HC even promoted pre-osteoblast proliferation independently of collagen origin. Then, cells were tested for differentiation parameters in the presence of HC. Only HC from bovin origin exhibited significant higher alkaline phosphase activity after 7 days of incubation when compared to its BSA control condition. This observation was supported by mineralization assays demonstrating that bovin HC enhanced Ca/P nodule formation in MC3T3-E1 cultures.

To confirm these encouraging results, C3H/HeN mice were ovariectomized (OVX) to induce bone loss and were given, in parallel, HC enriched diets to determine whether HC intake may contribute to bone health by preventing decrease in bone mineral density upon OVX. Diets were designed to contain 15% casein, 17.5% casein or 15% casein plus 2.5% HC from porcin, fish or bovin origin. As expected, OVX induced a dramatic loss of bone mineral density. However, although modest, HC from both bovin and fish origin exhibited a significant higher BMD than OVX control mice suggesting a nutritional protective role for HC and further supporting its potential benefits on bone health.

Mots-clés

Osteoporosis, Bone mineral density, cell culture, ovariectomized mice model

THE ESTABLISHMENT OF MICROFLUIDIC CO-CULTURE SYSTEM TO STUDY THE INTERPLAY BETWEEN SENSORIAL NERVOUS SYSTEM AND MESENCHYMAL STEM CELLS IN VIEW OF OSTEOGENESIS - P5

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Résumé

The existence of nerve fibers in bone and the expression of neural factors in close vicinity to bone cells have been reported. Neuron-derived molecules have demonstrated to play a role on bone turnover (1) and accumulating evidence has revealed the importance of the sensory nervous system in the orchestration of bone repair (2). The aim of this work is to establish a co-culture system between sensorial neurons and mesenchymal stem cells and to study the cellular interplay, between these cells, in view of osteogenesis.As to establish co-cultures between rat dorsal root ganglia (DRG) sensory neurons and rat bone marrow mesenchymal stem cells (MSCs) we fabricated PDMS microfluidic chambers, using standard photolithography techniques. This approach allows to physically separate cellular bodies from the distinct cell types to use two different cell culture media and to some extent to mimic the in vivo physiological environment (Figure 1A). The primary cells were co-cultured during 7 and 14 days in microfluidics system using DMEM (high glucose) with 1% B27 or DMEM with 10% FBS media for DRGs and MSCs, respectively. At the designated time points (i.e. 7 and 14days), metabolic activity, specific immunostaining and gene expression profiles (qPCR) were performed for cells in mono and co-cultures. We observed that during the time points tested the DRGs were able to spread neurites that transverse the microchannels and contact with MSC (see Figure 1B), as demonstrated by the specific CGRP staining. MSCs metabolic activity was not influenced by the co-culture with DRGs. Nonetheless, we could observe an impact on the expression of Runx2, a key gene for osteoblastic differentiation, when MSCs were co-cultivated with DRG neurons for 7 days. Other key genes for osteogenic differentiation were also evaluated, namely Osterix (Sp7) and osteocalcin (Bglap), and although a tendency for upregulation was established for 7 and 14 days, new studies are under way. Figure 1. (A) Co-culture microfluidics system. (B) Immunocytochemistry of DRG neurons (anti-CGRP antibody, green) and MSCs (phaloidine, red) co-cultures at 7 days of culture. Our preliminary results suggest that sensorial neurons can closely interact with MSCs and modulate their osteoblastic phenotype. This co-culture system will allow to broaden current knowledge on the impact of the sensorial nervous system for bone remodeling and repair, and can open new avenues for the establishment of new strategies for bone tissue regeneration.

Mots-clés

microfuidics system, sensorial neurons, bone, co-culture

Bioingeneering of mandibulare reconstruction in cancer surgery - P6

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Résumé

Worldwide 500,000 cases of head and neck cancer are diagnosed each year. Oral cancer is the sixth most frequent cancer in the world (1), representing 6,500 cases in France (2). Its treatment is based mostly on a combination of surgery, radiotherapy and chemotherapy. For tumor adjacent to / or infiltrating the mandible, resection of the mandible is the gold standard. This resection can be partial or segmental (3). After carcinologic surgery, the reconstruction of the mandibule is required and different techniques have been proposed: plates, graft, flap, distraction and more recently the technique described by Masquelet with the induced membranes. However, the technique described by Masquelet for orthopaedic indication exhibit limitations in a carcinological context: i) the nature of the induced membrane and its mechanical properties after radiation, ii) the use oof autografts and BMP2 for filling the chamber inside the bone defect. To improve this approach in such clinical situation, our project is to develop a new strategy for preparing a new induced and vascularized membrane in a first step of the surgery and to introduce inside the membrane in the second step, an injectable osteoinductive and osteoconductive composite polymer composed of nanohydroxyapatite (nHA) doped in strontium with polysaccharide-based scaffold, to stimulate bone formation and vascularization.The aim of the work presented here is to 1) synthesize nHA substituted by strontium with different ratio of strontium 2) associate these nanoparticules with polysaccharide-based scaffolds and 3) evalutate the fate of this composite polymer subcutaneously in mice.Regarding the methods: nHA particles has been prepared as previously described (15). For doped-nHA particles, different ratios of strontium nHA/polymer have been studied: 10 and 50 %. nHA particles has been characterized by X-Ray diffraction, ICP (Inductively coupled plasma mass spectrometry) and STEM (Scanning transmission electron microscopy). Then particles have been dispersed in polymer according to our previous work (15). Samples are implanted subcutaneously in 12 weeks BALB/c mice for 2 and 4 weeks. Micro-CT will be used for analyzis of the mineralization of the tissue, and histologically for bone formation analysis.Preliminary data suggest that the composite polymer developed with a ratio nHA-Sr 50%, is able to stimulate mineralization at an ectopic site. Histological analyses are under going to confirm the presence of osteoid and vascularized structures.In conclusion, this osteoinductive composite scaffold doped with strontium could be an alternative of the autografts supplemented with BMP2 for the regeneration of large bone defects in a carcinological context.

Mots-clés

Mandibular reconstruction - Hydroxyapatite - Strontium

The flavonoid fisetin promotes osteoblasts differentiation through Runx2 transcriptional activity - P7

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Résumé

Scope: Flavonoids represent a group of polyphenolic compounds commonly found in daily nutrition with proven health benefits. Among this group, the flavonol fisetin has been previously shown to protect bone by repressing osteoclast differentiation. In the present study, weinvestigated the role of fisetin in regulating osteoblasts physiology. Methods and results: In vivo mice treated with LPSs exhibited osteoporosis features associated with a dramatic repression of osteoblast marker expression. In this model, inhibition of osteocalcin and type I collagen alpha 1 transcription was partially countered by a daily consumption of fisetin. Interestingly, in vitro, fisetin promoted both osteoblast alkaline phosphatase activity and mineralization process. To decipher how fisetin may exert its positive effect on osteoblastogenesis, we analyzed its ability to control the runt-related transcription factor 2 (Runx2), a key organizer in developing and maturing osteoblasts. While fisetin did not impact Runx2 mRNA and protein levels, it upregulated its transcriptional activity. Actually, fisetin stimulated the luciferase activity of a reporter plasmid driven by the osteocalcin gene promoter that containsRunx2 binding sites and promoted the mRNA expression of osteocalcin and type I collagen alpha 1 targets. Conclusion: Bone sparing properties of fisetin also rely on its positive influence on osteoblast differentiation and activity.

Mots-clés

Bone / Fisetin / Osteoblast / Polyphenol / Runx2

OSTEOCLASTS ACTIVATE CHONDROCYTE CATABOLISM THROUGH S1P PRODUCTION - P8

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Résumé

Purpose: Subchondral bone features accompany all stages of osteoarthritis (OA). We have previously demonstrated that high osteoclastogenesis and bone remodeling is observed at the initiation of OA, while inhibition of osteoclast function prevents bone and cartilage catabolism in murine OA models. Our purpose was to evaluate how osteoclast-derived factors affect the chondrocyte metabolism and to further investigated the role of sphingosine 1 phosphate (S1P), an osteoclast-secreted molecule in chondrocyte metabolism and osteoarthritis. Methods:Primary murine chondrocytes were cultured with conditioned medium of osteoclasts (Oc-M) or RAW cells (Raw-CM) to analyze the expression of catabolism and anabolism genes (RT-qPCR). Femoral head explants were cultured in the presence of Oc-CM to quantify matrix protein expression and proteoglycan content and further investigate the role of S1P released in Oc-CM in the presence of JTE-013, a S1P receptor 2 (S1PR2) antagonist. Results:Oc-CM reduced the proteoglycan release in primary chondrocytes and activated MAPkinase pathway. Increased expression of catabolic enzymes (MMP-3, -13, Adamts-4,-5) was observed only with Oc-CM while reduction of expression of anabolic markers (Col2, ACAN, Sox9) was induced by both Oc-CM and Raw-CM. Oc-CM increased the chondrocytic expression of S1P receptors 1 to 4 and the inhibition of S1PR2 protected chondrocytes from degradation enzymes induced by Oc-CM. In joint explants, JTE-013 reversed proteoglycan loss and NITEGE expression induced by Oc-CM, and reduced proteoglycan release and expression of MMP-3 / MMP-13 by the chondrocytes. Our results indicate that S1P produced by osteoclasts promotes chondrocyte catabolism. Conclusion: These data demonstrate that osteoclast-secreted factors disrupt the balance of chondrocyte metabolism through the production of S1P. Therefore, subchondral bone manipulations may affect chondrocyte function and OA.

Mots-clés

Osteoarthritis, chondrocyte, osteoclast, sphingosine 1 phosphate

SILICON IN VEGETABLES: IN VITRO BIOACCESSIBILITY, BIOAVAILABILITY AND BIOLOGICAL ACTIVITY IN DIFFERENT TARGET TISSUES (INTESTINE AND BONE) - P9

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Résumé

Fruits and vegetables contain a number of components (polyphenols, carotenoids, vitamins, folate, minerals such as magnesium, calcium, silicon and other compounds) that could affect the bone health. Considering the biological activities induced by silicon (antioxidant activity, increased mineralization of bone, development of connective tissue), this study aims to compare the biological activity induced by the silicon biofortificated and unbiofortificated vegetables (tatsoi, mizuna, purslane, Swiss chard and chicory) respect to the commercial silicon supplement. In addition, the assessment of silicon bioaccessibility, bioavailability and biological activity in human intestinal cell line (Caco-2) and human osteoblasts (hOBs) were performed. After in vitro gastrointestinal digestion of plant edible leaves and the silicon supplementation, the silicon bioaccessibility ranged from 7.01 to 13.22 mg/L. On Caco-2 cell line, the antioxidant activity of the bioaccessible fraction was assessed as reduction of induced ROS levels and ranged from 25 to 44% respect to stimulated control. The silicon biofortification significantly ($p<0.001$) improved the antioxidant activity induced by the vegetables. The bioavailability of digested samples, evaluated on Caco-2 differentiated cell line, ranged from 237 to 440 μg/L with percentages ranged from 27% to 61% in relation to the considered vegetable species. The expression of collagen type 1 and alkaline phosphatase induced by the bioavailable fraction after 12 and 36 h of treatment was evaluated in hOBs by real-time PCR. The bioavailable fraction of tatsoi, purslane and Swiss chard (biofortificated leafy vegetables), improved (after 36 hours of incubation) the expression of collagen type 1 and alkaline phosphatase with respect to the silicon supplement and unbiofortifated vegetables in hOBs.

Mots-clés

bone, human intestinal cell lines, bioaccessibility, antioxidant activity, bioavailability, human osteoblasts

EFFECT OF THE LEUCINE RICH AMELOGENIN PEPTIDE ON ENAMEL STRUCTURE AND MINERALIZATION - P10

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Résumé

Dental enamel is the outer calcified tissue layer covering the crown of the tooth; it is the most mineralized tissue of the human body. Enamel is synthetized by the ameloblast during the course of tooth development but this cell population degenerates and totally regresses when the tooth becomes functional. The mature enamel is therefore completely devoid of cell and protein content. The Leucine Rich Amelogenin Peptide (LRAP) is a product of alternative splicing of the amelogenin gene, which is the main protein synthetized by the ameloblast. LRAP is a short peptide (56kDa) composed of the active N and C-terminal sequence of the amelogenin protein. This peptide has shown in vitro signaling properties toward odontoblast and ameloblast differentiation (Le et al., 2007, Tomkins et al., 2005). We have previously shown that the different forms of LRAP (with or without the phosphate group on Serine 16) exhibited a regulating effect on HAP crystal formation. The phosphorylated form (LRAP+P) inhibited HAP synthesis whereas the non-phosphorylated form (LRAP-P) exhibited a morphogenic effect on crystal growth (Le Norcy et al. 2011). These different studies suggest that LRAP could be a potential candidate for the development of therapeutic strategies against enamel defects.The aim of this work is to evaluate the biological effects of the different forms of LRAP on ameloblast differentiation and enamel formation. To this end, we have exploited two experimental models: 1) differentiation of the ameloblastic cell line LS8 in mineralizing conditions and 2) ex vivo cultures of mouse first mandibular molar germs withdrawn at postnatal day 0. Kinetic expression of genes involved in differentiation and mineralization of ameloblast is evaluated by qPCR, and TEM and SAED analysis of the mineral formed are completed. Development of the ex vivo germ culture is analyzed by histological staining and micro-CT imaging. After selection and isolation of the area of interest by Focused Ion Beam, mineral at the cusp tips is analyzed by TEM and SAED. The presence of both forms of LRAP influences the LS8 differentiation kinetics and activates in parallel enamel gene expression. Accordingly, an increase in the density and volume of mineral formed in the germ culture model is observed in presence of both forms of LRAP.This work aims to open new enamel tissue regeneration strategies in order to treat alteration in the enamel layer by decay or genetic disorders such as amelogenesis imperfecta.

Mots-clés

Biominéralisation - Email - Amélogénine - Hydroxyapatite

SEPARATION AND IDENTIFICATION OF THE OSTEOGENIC COMPOUNDS OF NACRE USING ION-EXCHANGE RESIN ASSOCIATED WITH A MINERALIZATION CELL MODEL - P11

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Résumé

Nacre, or mother of pearl, is constituted with aragonite (97%) and organics (3%)[1,2]. It is capable to increase the cell osteogenic activity[3] and has a brightly future in bone tissue engineering as a natural biomaterial. So far, osteogenic compounds of nacre have not been identified yet. We have confirmed the osteogenic potential of a particular nacre extract, ESM (Ethanol Soluble Matrix)[4]. In this context, we tried to identify the osteogenic compounds of ESM with ion-exchange resin. W e evaluated their mineralization induction capacity on osteoarthritis (OA) subchondral osteoblasts which are known to have difficulties to mineralize[5]. For this purpose, ESM was extracted from nacre powder of pearl oyster Pinctada margaritifera with ethanol. From ESM, cationic ESM (ESMc) and anionic ESM (ESMa) were achieved with the help of ion-exchange resin. Both were tested fo r 28 days at 200µg/ml on subchondral osteoblasts from OA patients undergoing total knee replace ment. Alizarin red staining was performed to visualize capacity of mineralization and quantified at 405 nm. Size-exclusion HPLC was also used to separate the molecules present in ESMc and ESMa. Our results with Alizarin Red assay demonstrated an increase of calcium deposition in OA osteobla sts in presence of ESMc from 21 days of culture. But in presence of ESMa no mineralization was o bserved. HPLC showed that ESMc and ESMa contain some molecules totally different in size. In co nclusion, ESM can be separated into 2 totally different fractions, ESMc and ESMa using ion-exchan ge resin. ESMa does not present any mineralization induction capacity on OA subchondral osteobl asts. All the osteogenic compounds present in ESM are cationic. We believe that our strategy asso ciating ion-exchange resin, OA osteoblasts, cation-exchange HPLC and mass spectrometry could b e effective to identify nacreous osteogenic molecules. This improvement will advance indirectly th e use of nacre in bone tissue engineering.

Mots-clés

Osteogenic compounds of nacre, separation and identification, ion-exchange resin, osteoarthritic osteoblasts, mineralization, HPLC

Bone loss in early phases of rat arthritis is predictive to disease severity - P12

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Résumé

Objectives. Hallmarks of rheumatoid arthritis (RA) are joint synovitis and subchondral bone erosions, resulting in loss of joint function. While synovial inflammation can be easily assessed, occurrence and kinetics of periarticular bone loss remain unclear in RA, especially in early phases, essential to understand and prevent arthritis. So, we used the rat adjuvant-induced arthritis (AIA) model in a longitudinal study to characterize finely bone loss kinetics for 4 different bones in inflamed ankles (the prominent sites in this model) along time, especially around arthritis onset. Methods. Ten 6-weeks-old female Lewis rats were injected with Mycobacterium butyricum to induce arthritis (AIA) while 10 control rats received only mineral oil solution. Several clinical parameters, including articular index (AI), were monitored during the experiment. Bone microarchitecture and bone mineral density (BMD) profiles were obtained by µ-CT and DXA, respectively, at day0, at the arthritis onset, and at day17. Local sites analyzed included the main four ankle bones: navicular, talus, cuboid, and calcaneus bones. Systemic sites enclosed tibia and 2nd lumbar vertebra (LV2). Bone histology was performed at day of sacrifice (day17).Results. Arthritis onset occurred at day10, with different levels of arthritis. At day10, early microarchitecture changes were detected in the four ankle bones analyzed in AIA compared to control rats. They included decreased trabecular bone volume and thickness ($p<0.01$) and increased cortical porosity ($p<0.01$). At day17, extended ankle bone changes were assessed by µ-CT and correlated with histology assessment. Bone mineral density (BMD) was highly decreased in AIA rats ankle compared to control rats (-22%; $p<0.001$). There was also a systemic BMD decreased in AIA rats compared to control rats (tibia -16%, LV2 -15%; $p<0.001$ for both). In AIA rats, ankle-tibia-LV2 BMD values were correlated together (r from 0.77 to 0.87; $p<0.001$ for all), while BMD values were randomly distributed in control rats. In AIA rats, early bone microarchitecture changes correlated with arthritis severity at day17, in both synovial inflammation and bone loss parameters (for instance, bone volume at day10 and AI at day17, $r=-0.90$, $p<0.001$). Conclusion. Bone microarchitecture changes at arthritis onset in the rat AIA model are observed in four bones of ankle, suggesting a precocious role for bone compartment in arthritis, together with the well-described synovial inflammation. These early bone changes are correlated with arthritis outcome at day17, in terms of synovial inflammation, local joint destruction, and systemic bone loss, strengthening importance of early bone monitoring in this disease.

Mots-clés

arthritis, bone loss, bone microarchitecture

A new therapeutic approach for achondroplasia and hypochondroplasia: Tyrosine Kinase Inhibitor “BN016” - P13

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Résumé

Missense mutations localized in FGFR3 (Fibroblast Growth Factor Receptor 3) gene lead to a family of dwarfisms with a spectrum of severity. Most frequent diseases for this family are Achondroplasia (ACH) and Hypochondroplasia (HCH). Fgfr3 mutations induce an increased phosphorylation of this tyrosine kinase receptor correlated with an enhanced activation of its downstream signaling pathways. A strong endochondral ossification defect is one of the consequences of the FGFR3 gain of function mutation. Nowadays several preclinical studies have been carried out (CNP analog BMN111, intermittent PTH injections, soluble FGFR3 therapy, statin treatment). Since these diseases are due to FGFR3 over-activation, the use of Tyrosine Kinase Inhibitors (TKIs) to arrest FGFR3 phosphorylation seems to be a good therapeutic target. TKIs are a class of molecules ATP-mime that are able to interact with its protein binding site without establishing any phosphorylation. For this reason we performed experiments to evaluate the action of a new TKI “BN016”. We performed in vitro studies in immortalized human chondrocytes transiently transfected with FGFR3 WT or mutant. BN016 reduces FGFR3 phosphorylation, modifies FGFR3 protein glycosylation and inhibits FGFR3 downstream pathways (MAPKs). We evaluated the effect of BN016 on a Fgfr3 mouse model expressing heterozygous mutation Y367C that mimics main clinical features of ACH phenotype. We performed ex vivo studies on 16.5 dpc embryos of Fgfr3 (Y367C/+) mice showing that we can rescue femur phenotype using BN016 at 100 nM. Immunohistological analyses have been performed showing the rescue of growth plate organization and cell differentiation in treated femurs. We also performed in vivo experiments: Fgfr3 (Y367C/+) mice have been daily and subcutaneously injected from P1 to P15 with 2 mg/kg of BN016 and compared to untreated controls. Mice were sacrificed at P16 and bones were collected. We observed an overall increase of bone length in treated mice: tail (+21%), anteroposterior skull axis (+9%), femur (+16%), tibia (+20%), humerus (+9%), ulna (+16%) and radius (+12%, caliper measurements). Immunohistological analysis for BrdU showed an enhancement in cell proliferation at the growth plate level in treated mice. Histological analyses showed that BN016 leads to a correction of the disturbed growth plate. µCT analyses confirmed the results on bone length (femur +19%, tibia +21%): we observed an increase of distal femur (62%) and proximal tibia (71%) growth plate height. In conclusion our findings support the idea that TKIs could be a therapeutic tool for improvement of the growth in ACH and HCH.

Mots-clés

dwarfism, FGFR3, tyrosine kinase inhibitor, therapy

DEVELOPPEMENT D'UN MODELE D'OSTEOGENESE IN VITRO AU SEIN DE MATERIAUX 3D - P14

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Résumé

In vivo, les cellules évoluent dans un microenvironnement complexe, mais surtout tridimensionnel. Cependant, la majeur partie de nos connaissances sur la physiologie cellulaire in vitro a été obtenue à partir de cultures de cellules faites en boites de Pétri, donc en 2 dimensions. Ces conditions différentes ne peuvent qu'avoir une influence sur le comportement des cellules, qui n'entretiennent plus les mêmes relations spatiales entre elles, ainsi qu'avec leur environnement. Le développement de modèles de culture cellulaire osseuse en trois dimensions (3D) permettrait donc de se rapprocher de l'environnement in vivo des cellules, et ainsi de mieux comprendre les différents aspects de leur fonctionnement .Afin de recréer une ostéogénèse in vitro dans un environnement proche du tissu osseux, nous utilisons des biomatériaux macroporeux en hydroxyapatite, cultivés sous perfusion dans le bioréacteur Bose ElectroForce® 5270 BioDynamic®. Ces biomatériaux cylindriques, traversés par des canaux de différentes géométries, assurent une bonne perfusion de la culture. Outre une architecture circulaire, les conduits peuvent également présenter une géométrie triangulaire, ce qui permettra d'étudier l'impact de structures en « V » sur la différenciation des progéniteurs ostéoblastiques. La mise au point des cultures 3D dans le système Bose a été réalisée sur des matériaux modèles en plexiglas (PMMA), possédant la même architecture que les biomatériaux en phosphate de calcium à pores circulaires, qui constituent le substrat de référence pour notre l_c0étude. Les systèmes d'ensemencement et de perfusion ont été optimisés, ce qui a permis d'obtenir une répartition ainsi qu'une croissance cellulaire homogènes au sein du matériau. Le flux de perfusion permet aux cellules de croître pendant deux à trois semaines. La coloration de la matrice minéralisée par le rouge Alizarine a permis de confirmer qu'un processus d'ostéogénèse se déroule au sein du matériau. La culture dans le système Bose, bien adaptée à la mesure en ligne des paramètres physico-chimiques de l'environnement cellulaire (température, pression partielle en O2), permettra, en y associant le dosage de marqueurs du métabolisme osseux, de réaliser un suivi continu de l_c0évolution des cultures osseuses 3D, ainsi que de leur réponse aux hormones et/ou agents pharmacologiques. Outre des avancées dans la compréhension de la biologie de la physiopathologie de l'os, ce modèle pourra donc aussi être utilisé pour le test de nouvelles molécules ciblant le remodelage et le métabolisme osseux.

Mots-clés

Ingénierie tissulaire, biomatériaux, hydroxyapatite, ostéogénèse

DISTINCT EXPRESSION OF IL-36α, β, γ AND THEIR ANTAGONISTS IN RHEUMATOID ARTHRITIS - P15

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Résumé

The IL-1 family comprises 11 proteins including three recently discovered cytokines encoded by three different genes: IL-36α, β and γ. These molecules bind to the same receptor IL-36R, inducing signaling via NFkB and MAPK pathways and thus amplification of inflammation and immune responses similar to IL-1α/β. IL-36RA is the specific antagonist of IL-36 cytokines. One study demonstrated that IL-38 is also able to bind and neutralize IL-36R. IL-36α, β and γ are over-expressed in psoriatic lesional skin, are induced by inflammatory cytokines in keratinocytes and have a key role in psoriasis pathophysiology. In contrast, IL-36 cytokines have no significant role in different arthritis mouse models. Nonetheless, IL-36α is highly expressed by plasmocytes in the synovial membrane and IL-36β is over-expressed in the serum of rheumatoid arthritis (RA) patients. Here, to better understand this discrepancy and because no comparative and complete study exists about expression of IL-36α, β, γ and their antagonists in RA, we analyzed their relative expression and cell sources in RA patients as well as in several mouse arthritis models. By RT-qPCR, we show that IL-36 cytokines and their antagonists were over-expressed in the joints of three different mouse models of arthritis but at different levels and time frames. By immunohistochemistry (IHC) on mouse joint sections, IL-36b was found in synovial macrophages and multinucleated cells eventually closed to the bone but not in normal osteoclasts. By ELISA, IL-36α, β, γ and their antagonists were found significantly over-expressed in the synovial fluids of RA versus osteoarthritis (OA) patients, but again at very different levels. By IHC and multiple fluorescent labeling on RA patients' synovial membranes, IL-36α, β and γ were found in CD79α+ plasmocytes but also in different populations of CD68+ macrophages or dendritic cells. To confirm these results, primary cultures of fibroblast-like synoviocytes (FLS) and CD14+ monocytes differenciated into M1 (inflammatory macrophages), M2c (anti-inflammatory macrophages), dendritic cells (antigen presenting cells) and osteoclasts (bone resorbing cells) were analyzed. Inflammatory macrophages and dendritic cells appeared as the major cell sources of IL-36α, β and γ. However, these cytokines were differently expressed and were induced by distinct stimuli such as IFNγ or lipopolysaccharides. Overall, these results suggest that IL-36α, β, γ and their antagonists IL-36RA and IL-38 are produced by different cell types and in distinct pathophysiological conditions. Because several specific patterns of expression were found in patients, these results raised the possibility that only a sub-group of RA patients would benefit from IL-36 neutralization.

Mots-clés

Rheumatoid arthritis, interleukine 36 alpha, beta, gamma, IL-36RA, IL-38

CYSTIC FIBROSIS BONE DISEASE: AN ELEVATED RANK-L / OPG PROTEIN RATIO IN OSTEOBLAST WITH THE F508DEL-CFTR MUTATION - P16

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Résumé

Cystic Fibrosis (CF) is the most common lethal genetic disease in Caucasian population, which is caused by mutations in gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. F508del is the most common CFTR mutation, with more than 80% of patients carrying it on at least one allele. Osteoporosis and increased vertebral fracture risk associated with CF disease are becoming more important as the life expectancy of patients continues to improve. This further translated to a 100-fold greater risk of vertebral compression, which can decrease lung function, thus accelerating the course of the disease and decreasing the patient's quality of life. Although osteoporosis is commonly attributed to increased bone resorption due to the chronic inflammatory status of CF patients, physiopathology of the CF-related bone disease remains unknown; and others cells like osteoblasts (cells that form bone) can be affected by the CFTR mutation. We especially study the impact of the most represented mutation in cystic fibrosis, F508del-CFTR, on the osteoblastic function. Mineralization profile of primary human osteoblasts bearing the ΔF508del CFTR mutation (CF patients, n=4) or not (non-CF patients, n=4) were followed during 21 days in culture by using alkaline phosphatase (ALP) activity and calcium deposition measurements. CF osteoblasts' ALP activity was found to be higher compared to that observed in non-CF osteoblasts and stay stable for the 14 days-period. We also observed an increase in calcium deposition that started sooner in CF than in non-CF osteoblasts. Serum levels of cytokines TNF-α and IL-17 are elevated in patients with CF [2]. We therefore analysed the effect of TNF-α and IL-17 stimulation on the secretion of both RANK-L and OPG protein by CF osteoblasts. In resting conditions, we showed that the RANK-L/OPG protein ratio was greatly increased in CF osteoblasts compared to non-CF osteoblasts, in correlation with our previous results showing high RANK-L/OPG mRNA levels [3]. This imbalance was more pronounced under TNF-α stimulation but not under IL-17 stimulation. Our data suggest that in CF bone disease, the increased RANK-L/OPG ratio observed in osteoblasts with the F508del mutation may modify the bone formation/resorption equilibrium. That dysregulation may be also amplified in CF patients under a chronic inflammatory state.

Mots-clés

Cystic fibrosis ; bone disease ; primary human osteoblasts ; mineralization ; RANK-L/OPG

Glycosyl-Nucleosyl-Fluorinated - Collagen injectable hydrogel for tissue engineering: A new scaffold for bone regeneration. - P17

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Résumé

Bone is a dynamic and vascularized tissue that has the ability of naturally healing when damaged. Nevertheless, when large bone trauma occurs, bone loses this ability. Bone Tissue Engineering (BTE) has been emerging as an approach for bone regeneration. This strategy is based on the use of three dimensional (3D) matrices to guide both cellular proliferation and differentiation. In previous work our group has shown that a porous 3D hydrogel could be prepared by the self-assembly of the low molecular weight amphiphilic monomer Glycosyl-Nucleoside-Fluorinated (GNF). Several biological tests have already showed that the GNF gel was: 1) non-cytotoxic to clustered human Adipose derived mesenchymal Stem Cells (hASCs), 2) bioinjectable and 3) linearly biodegradable. Although hASCs cannot adhere to the gel, preformed cell aggregates proliferate and differentiate normally when entrapped in the GNF-based gel [1]. However, GNF requires to be coupled with another protein matrix in order to increase cytocompatibility property. Thereby, the overall aim of this work is to design, develop and validate a new composite hydrogel for BTE, composed of GNF and a protein matrix well known for its cytocompatibility: collagen [2]. The collagen-GNF hydrogel is expected to lead to a homogenous dispersion and a stable frame, which will improve viability and proliferation of cells within the hydrogel. In particular, the goals of this study were to investigate: 1) the effects of the association of collagen to GNF regarding cell adhesion and proliferation and 2) to study the stability and integrity of the gels, due to the superior rheological properties of GNF, in comparison with collagen alone. Rat tail collagen (4mg.ml-1) was prepared in our laboratory [3] and blended with GNF at different GNF concentrations: 0,5; 1,5; 3 and 5% (weight/volume) before gelation. Our results show that the addition of collagen to GNF increases cellular metabolic activity within the hydrogel. Additionally, and as expected, collagen gels seeded with cells shown to retract and reduce in volume within a few days. However, when GNF was added, the hydrogel retained its shape and its original volume during the time scale of the study (15 days). We also evaluated cell distribution and viability within the hydrogel and shown that collagen-GNF blend presents the potential for the promotion of cell adhesion, spreading and proliferation. In conclusion, GNF-Collagen based gels are a promising novel class of hydrogels for BTE applications.

Mots-clés

bone tissue engineering hydrogel scaffold

Un vol spatial d'un mois à bord du bio satellite russe BION M1 fragilise sévèrement le squelette des souris - P18

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Résumé

Après 16 ans d'interruption, les vols spatiaux consacrés à la recherche biomédicale embarquant des rongeurs ont repris. Avec succès, plusieurs groupes de souris ont décollé de Baïkonour le 19 avril 2013, ont séjourné dans le bio satellite russe Bion-M1 à 570 km d'altitude avant de redescendre sur terre un mois plus tard. Profitant de l'opportunité unique d'analyser le tissu osseux de ces souris, ce projet s'attache à étudier l'influence de la gravité sur le squelette murin.Pendant ces 30 jours, 3 groupes de 5 à 6 souris mâles C57/Bl6 âgées de 15 semaines ont soit séjourné dans l'espace (Flight), soit sont restées sur terre dans des conditions d'hébergement synchrones (cages, nourriture, température (Syn)), soit sont restées sur terre dans des conditions d'hébergement standard (Ctr).Dans les fémurs, les animaux Flight et Syn ont des paramètres de micro-architecture osseuse considérablement fragilisés tels que le BV/TV (%, moy±SEM) (Flight: 1.69±0.4 ; Syn: 4.80±0.89 ; Ctr: 11.4±1.72 ; $p<0.05$) ou l'épaisseur corticale (µm, moy±SEM) (Flight: 194±4.8 ; Syn: 205±2.7 ; Ctr: 215±3 ; $p<0.05$). Au niveau cortical, nous observons dans le groupe Flight une résorption périostée, une expression ostéocytaire de Sost augmentée et une désorganisation des lacunes ostéocytaires. On observe également des altérations de la qualité intrinsèque du tissu osseux cortical chez les animaux du groupe flight vs Ctr ; les valeurs des paramètres caractérisant les propriétés plastiques et élastiques mesurées par nanoindentation sont diminuées. Dans la vertèbre T12, le BV/TV (%, moy±SEM) est également diminué (Flight: 22.9±1.9, Syn: 28.5±0.63 Ctr: 30.9±1.46 ; $p<0.05$). Dans la vertèbre L3, la résorption est accrue (Oc.S/BS: %, moy ±SEM) (Flight: 0.62±0.12 ; Syn: 0.13±0.03 ; Ctr: 0.11±0.01 ; $p<0.01$ entre le groupe Flight et les autres groupes). Dans la vertèbre L2, les premières investigations FTIRM (Microspectroscopie Infrarouge par Transformée de Fourier) révèlent un défaut de maturité du collagène dans les groupes Flight et Syn vs Ctr. En revanche, le degré de minéralisation évalué par microradiographie n'est pas modifié. Les distances intervertébrales, mesurées sur les reconstructions tomographiques du rachis thoracique postérieur, n'ont pas été modifiées ni par la microgravité, ni par les conditions synchrones. Des analyses complémentaires sont actuellement en cours pour mieux comprendre les mécanismes de perte osseuse et de récupération lors du retour sur terre après un vol spatial. Néanmoins, nos premiers résultats révèlent une contribution majeure de la gravité dans le maintien de l'intégrité du squelette.

Mots-clés

Microgravité, Souris, Squelette

INSIGHT INTO THE EXTRACELLULAR PHOSPHATE SENSING MECHANISM, THE KEY STEP FOR AN APPROPRIATE FGF23 SECRETION - P19

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Résumé

Phosphate (Pi) is known to be a vital ion involved in structural and metabolic functions. More recently, several studies have suggested that Pi is a signaling molecule. Notably, we have shown in pre-osteoblasts (MC3T3-E1), in chondrocytes (MC615) and in odontoblasts (MO6-G3) that an increase in extracellular Pi concentration leads to the ERK1/2 pathway activation. This pathway notably allows the up-regulation of genes involved in bone mineralization. However, the mechanism underlying the sensing of extracellular Pi variations remains still unclear. Recently, we have shown in in vitro experiments with MC3T3-E1 and MC615 cells that both PiT1 and PiT2 proteins are likely to be involved in this process. PiT proteins are multifunctional proteins originally described as retroviral receptors and sodium-Pi cotransporters. Additional experiments have shown that PiT2 could oligomerize in response to extracellular Pi variations. Considering the strong homology between PiT1 and PiT2 we hypothesize that a PiT1-PiT2 interaction could underlie the mechanism whereby the ERK1/2 pathway is activated. To investigate this putative interaction, we are embarking in Bioluminescence Resonance Energy Transfer (BRET) experiments. To this end, we have fused the coding sequence of YFP or Renilla luciferase on PiT proteins using the Fast Cloning method. To assess the interaction of PiT1 and PiT2 proteins, we are also using co-immunoprecipitation upon Pi stimulation with a BS3-based crosslinking approach. In vivo, an increase in serum Pi concentration was shown to stimulate the secretion of Fibroblast Growth Factor 23 (FGF23). FGF23 is a phosphatonin produced and secreted by the osteocytes that tightly regulates the Pi homeostasis. Therefore to study the physiological relevance of the involvement of PiT proteins in Pi sensing, we have developed new mouse models carrying specific PiT1 and/or PiT2 deletions in osteocytes. To this aim, we have generated PiT1lox/lox, PiT2lox/lox and PiT1lox/lox;PiT2lox/lox mice using homologous recombination, and crossed them with Dentin Matrix Protein 1 (DMP1)-Cre transgenic mice. When the Pi food supply is normal, the gross phenotype of mutant mice is not affected. However, feeding mutant mice with low- or high-Pi diets resulted in aberrant regulation of FGF23 secretion. This study will unravel the molecular mechanisms whereby Pi informs the cell of its own extracellular variation which will lead to an appropriate FGF23 secretion resulting in a normal regulation of Pi homeostasis.

GENERATION OF NUCLEUS PULPOSUS PROGENITOR CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS - P20

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Résumé

Introduction: The nucleus pulposus (NP) is the central part of the intervertebral disc (IVD) that plays a key role in spine kinematic. Recent lineage tracing studies have shown that NP cells are derived from the embryonic notochord. This continuous structure of mesendodermal cells plays a central role in axial skeleton formation. During IVD maturation, notochord cells (NTCs) that are considered the NP progenitor cells, progressively disappear. This process is probably the primary event leading to further NP degeneration. The generation of NTCs is therefore a promising approach to regenerate IVD. In this study, we examined the capacity of human induced-pluripotent stem cells (hiPSCs) to differentiate towards the notochord lineage. Methods: Human dermal fibroblasts were reprogrammed by mRNA encoding OCT4, SOX2, MYC, KLF4, NANOG and LIN28 transcription factors. The expression of pluripotency markers NANOG, SOX2 and OCT4, the integrity of karyotype and teratoma formation were analysed to assess the pluripotency state of reprogrammed cells. The Activin/Nodal pathway is required for pluripotent cells commitment towards mesendoderm progenitors. Thus, for differentiation, hiPSCs were treated for 2 days with 10ng/ml of activin A and then cultivated for 5 additional days without activin A. The efficiency of hiPSCs commitment was evaluated at day 2 by analyzing the FOXA2+/T+ cell fraction. A time course analysis was done during the differentiation to assess the acquisition of notochord (FOXA2, T, NOTO) and endoderm (SOX17) cell fate. Results: Our data show that reprogrammed fibroblasts expressed pluripotency markers, displayed a normal karyotype and formed teratomas composed of cartilage tissue (mesoderm), hair bulb structures (ectoderm) and epithelium with mucus (endoderm). Our data show that the activin A treatment induced around 10% of FOXA2+/T+ cells at day 2. After 2 days of induction, these mesendoderm progenitors persistently expressed high levels of FOXA2 and low levels of SOX17. The expression of NOTO was detected from days 2 to 4. Nevertheless, the stable expression of pluripotency markers indicated that activin A treatment was not sufficient to induce a robust commitment towards notochord lineage.Conclusions: We successfully generated integration free hiPSCs to overcome safety concerns for cell therapy. We demonstrated that these cells are able to differentiate towards mesendoderm. We are currently investigating whether the activation of Wnt pathway will increase the efficiency and specificity of the differentiation system towards notochord lineage.

Mots-clés

pluripotent stem cells, mesendoderm, differentiation

Osteoblastic syndecan-2 is a new orchestrator of Wnt signaling in bone cells - P21

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Résumé

Wnt modulators are potent targets for therapeutic approaches to manage skeletal diseases. Wnt factors but also the agonists R-spondins bind to glycosaminoglycan chains of syndecans that hence control their distribution in the cell microenvironment. Syndecans are therefore potent local modulators of Wnt signaling. Syndecan-2 expression occurs during osteoblast differentiation and is high in mature osteoblasts but its function was unknown. Here, we investigated the interactions between syndecan-2 and Wnt signaling using transgenic mice overexpressing syndecan-2 under the control of the 2.3 Kb fragment of collagen I promoter (Tg). The adult Tg mice had a lower bone mass, smaller osteoblastic and osteoclastic surfaces and reduced bone formation rate compared to wild type (Wt) mice. Mechanistically, Wnt target genes such as Axin, WISP and R-spondin-2 (RSPO2) were decreased in osteoblasts and marrow cells derived from Tg bones as compared to Wt, suggesting that syndecan-2 is a negative modulator of the transcriptional activity of beta-catenin/TCF. Immunohistological analyses confirmed the low levels of RSPO2, showed a strong decrease in Wnt receptors Frizzled (FZD) and an accumulation of the FZD-ubiquitin-ligase ZNRF3 at the surface of bone cells of Tg mice. Stimulation with recombinant Wnt3a resulted in an increase of RSPO2 and FZD into the cytoplasm of Wt bone-derived osteoblasts or C3H10 cells and in a further decrease of these compounds in syndecan-2 overexpressing cells. Wnt3a treatment also induced RSPO2 ubiquitination in Syndecan-2 overexpressing cells, suggesting that the proteoglycan was responsible for RSPO2 clearance from bone cells. An intriguing observation was that not only osteoblasts but also marrow cells displayed low RSPO2, FZD and LRP6 expression in Tg mice, suggesting that osteoblastic syndecan-2 regulates Wnt signaling in neighboring cells. In support to this hypothesis, using porous inserts to co-culture osteoblasts derived from Wt or Tg mice with C3H10 cells transfected with the TOPFlash reporter, we showed that syndecan-2 overexpressing osteoblasts reduced canonical Wnt pathway in distant cells. Conditioned medium on syndecan-2 overexpressing osteoblasts did not have the same inhibitory effects indicating that modulation of Wnt signaling by syndecan-2 did not depend on compounds released from syndecan-2 overexpressing cells.Altogether, our results show that high levels of osteoblastic syndecan-2 are associated with a decreased bone mass. Syndecan-2 induces R-spondin suppression through decreased TCF-dependent transcription and increased proteasomal degradation. This results in FZD-ubiquitin-ligase stabilization and Wnt signal extinction. Our results also reveal a new role of Syndecan-2 in the microenvironment of bone marrow cells as Wnt signaling orchestrator.

Mots-clés

syndecan-2, Wnt signaling, bone cells

A NEW ES CELL LINE AS AN ALTERNATIVE SOURCE FOR NEURAL CREST DERIVED STRUCTURES IN TISSUE ENGINEERING - P22

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Résumé

Understanding the embryonic development and mineralization of maxillofacial structures is crucial for developing innovative techniques for tissue engineering therapeutic approaches. Neural crest cells (NCC) are a multipotent and migratory cell population unique to vertebrates that gives rise to diverse cell lineage including melanocytes, smooth muscle, peripheral and enteric neuron, glia and the mesenchymal tissues of the tooth. In the context of tissue engineering applications, the proof of concept of the generation of a tooth has been made in the mouse, using ED14 dental epithelium and ectomesenchymal neural crest-derived cell reassociations, including tooth implantation in the jaw (Oshima et al., 2011). However, for obvious ethical reasons, a transfer of these data to the human absolutely required the identification of alternative cell sources. Pluripotent stem cells differentiated towards a dental ectomesenchymal program would represent an attractive alternative in the context of tissue engineering. For now, the best system for efficiently testing a cell odontogenic potential is set up in the mouse (Keller et al., 2013) and thus murine pluripotent stem cell have to be used as a start. In view of obtaining such a cell population, we have derived an ES line from blastocysts of a cross between Wnt1-CRE and RosaTomato mice. Since these ES cells expressed the fluorescent Tomato protein as they acquire the neural crest identity (NC), which allows for easily following their differentiation as well as its homogeneity. We then have set up an efficient differentiation protocol towards a NC phenotype (2D culture and defined medium). qPCR, immunofliuorescence and histological analyses have allowed to show that 1) this new ES Wnt1Cre RosaTomato cell line displays all the classical characteristics of an ES cell i.e. normal karyotype, capacity to differentiate in vitro towards derivatives of the 3 embryonic layers and to form teratoma. 2) upon induction in our NC differentiation protocol, it acquires the NC characteristics markers in parallel with the tomato fluorescence. This new cellular tool should help in unravelling the signals involved in the acquisition of the odontogenic potential by the dental ectomesenchyme, as well as, to carry out fate analysis in NC development and tissue engineering.

Mots-clés

Stem Cell characterization, Neural crest cell differentiation, WNT1/CRE Tomato ES cell line.

A SYSTEMATIC REVIEW ON PRE-CLINICAL AND CLINICAL APPLICATIONS OF RAPID PROTOTYPING IN BONE TISSUE ENGINEERING - P23

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Résumé

Introduction: Typical tissue engineering approach requires the combination of cells specific to the target tissue, growth factors and a biocompatible scaffold. Scaffolds for bone tissue engineering (BTE) can be prepared with natural or synthetic biomaterials and they must possess specific features, regarding pore diameters, porosity and macroscopic dimensions. There are different techniques to fabricate scaffolds for BTE and rapid prototyping (RP) using additive technologies are of growing interest since they allow custom tridimensional scaffolds production at high resolution. The aim of this systematic literature review was to evaluate the use of Additive RP to build scaffolds for pre-clinical and clinical applications in oral and maxillofacial surgery.
Methods: The search was performed on Pubmed database on the 10 past years, using specific key words: “rapid prototyping” or “scaffold” in combination with “oral surgery” or “maxillofacial surgery” and “implantation”. Inclusion criteria were "scaffolds made by an additive RP method" and "application in oral/maxillofacial surgery". In order to be included, the articles had to possess at least one of these two criteria. The experimental reports without scaffolds were excluded, as well as those that did not follow our including criteria. Reviews were used to search cross-references manually.
Results: 40 articles were selected, including case reports. Eight different RP methods for scaffold fabrication were used but 3D printing (11 articles) and Fused Deposition Modeling (7 articles) were mostly used. The effect of 3D organization of cells and scaffold was investigated in vivo. Preclinical models were based on different species including mice, rats, rabbits, dogs, sheep and pigs. Five studies were performed in patients for the reconstruction of mandibular ridge or angle and maxillary ridge or sinus. Cells were used in 26 studies to enhance tissue formation inside the scaffolds: MSCs of human or animal origin were used in 14 articles, alone or combined with other cell types (HUVECs) or growth factors (BMP2, BMP7, TGF-β2 or VEGF). The main advantages of RP scaffolds were 1) Obtaining of uniform porosity and pre-defined architecture and 2) Large amount of biodegradable and biocompatible materials available.
Discussion: Any shape and detail can be designed by CAD/CAM software and then fabricated by additive RP, so the pore diameter and scaffold porosity can be perfectly controlled. RP allows the use of biodegradable materials with a sufficient mechanical strength to be used for Bone Regeneration.

Mots-clés

rapid prototyping / scaffold / oral surgery / maxillofacial surgery / implantation

SHORT-TERM EFFECTS OF ZOLEDRONIC ACID ON BONE QUALITY AT MOLECULAR LEVEL IN THE RAT MANDIBLES - P24

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Résumé

Introduction: Bisphosphonates (BPs) are anti-resorptive drugs used in the treatment of benign and malignant bone diseases. Despite a recognized clinical efficiency, rare side effects have been reported following long-term administration of these molecules (e.g. osteonecrosis of the jaws, atypical fractures). BPs bind preferentially in bones having strong remodeling activity and have a high affinity for the mineral phase (1). However, their impacts on the bone composition and molecular structure are still poorly understood, especially in the mandible (2,3). The purpose of this work is thus to analyze short-term effect of zoledronic acid on bone quality according to the tissue age and site of the rat mandible by Raman microspectrometry. Material and Methods: Two groups of adult male rats (Sprague-Dawley) received 6 weekly intraperitoneal injections of either serum (S, n=15) or zoledronic acid (100 µg/kg/week, ZA, n=15). Double labeling was performed to distinguish newly formed bone from old bone (calcein, demeclocycline, 30 mg/kg). Right hemi-mandibles were harvested and embedded in resin to perform undecalcified sections through the first molar. Old bone and newly formed bone were revealed by fluorescence microscopy. Raman analyses were performed on bone as function of tissue age and site. The following Raman physico-chemical parameters (PPC) were calculated: mineralization (960/1450), crystallinity (1/FWHM), carbonation (1070/960), mineral maturity (1004/960), hydroxyproline/proline (879/854) and collagen cross-links (1660/1690). PPC values were compared according to the treatment(S vs ZA), the tissue age (new vs old) and site (alveolar vs basal).Results: At equivalent tissue age and site, PPC were not modified by ZA treatment compared to control. However, independently of the treatment, PPC were modified as function of the tissue age and site. Indeed, in the older bone, the mineralization ratio (+35%, p=0,001), crystallinity (+15%, p=0,001) and hydroxyproline-proline ratio (+9%, p=0,001) were increased and the HPO4/PO4 ratio (-35%, p=0,001) was decreased compared to the younger bone. In addition, for the same tissue age, the basal bone exhibited higher crystallinity (+2%, p=0,01) and hydroxyproline-proline ratio (+3,5%, p=0,05) than the alveolar bone, indicating a differential tissue maturation.Conclusion: The present study shows that newly formed bone quality assessed at the molecular level was unchanged by short-term ZA treatment neither in alveolar nor in basal bone. Additionally, this work provides information about the differences related to the tissue age and histological site of the mandibular bone. In particular, the alveolar bone exhibited less mature crystalline and collagenic molecular organization than basal bone at equivalent tissue age.

Mots-clés

zoledronic acid, bone quality, Raman spectrometry

NAMPT IS REQUIRED FOR OSTEOBLAST'S ACTIVITY AND OSTEOCLASTOGENESIS IN BONE REMODELING - P27

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Résumé

Nampt is limiting enzyme implicated in NAD metabolism and exerts growth factor and cytokine activity. Nampt is required for mesenchymal stem cells differentiation into osteoblasts, increases proliferation and matrix production in M3C3-T1 and suppresses osteoclastogenesis. Today, we lack data on role of nampt in bone remodeling. Indeed, nampt produced NAD, majorly consumed by Sirtuin (Sirt) and Sirt-1 is regulator of bone mass. Moreover, nampt expression is upregulated in periodontitis and rheumatoid arthritis and blocking its activity in experimental arthritis prevents from bone degradationAim: to study expression and role of nampt in bone remodeling. Material and methods: in vivo, protein expression in alveolar bone remodeling is studied using immunohistochemistry. Nampt activity is inhibited using FK866. Primary murine calvaria osteoblasts are cultured in non-osteogenic (OG) and OG media. Cells are challenged using recombinant nampt or NMN, first product of nampt. mRNA expression and protein is analyzed respectively using real-time RT-PCR and immunoblot. Results: 1- Nampt mRNA and protein expressions are significantly increased with osteoblasts differentiation cultured in 3 weeks in non-OG and OG media (x3.1, p≤0.01 and x4.4, p≤0.05 respectively at 2 & 3 weeks of culture). 2- 10µM NMN significantly induced expression of late markers of osteoblasts differentiation (BSP: x7.1; OC: x2.1, p≤0.05). 3- 1nM FK866 significantly inhibited late markers of osteoblasts differentiation (BSP and OC : 59% decrease, p≤0.05) 4- 0.2 – 5 µg/ml nampt for 6h induced increased RANKL/OPG ratio and MMP-13 mRNA expression (x10 and x4 respectively) . 5- Basal expression of nampt in periosteum OG layer increased at 18h after induction of bone remodeling (x 2.7 fold, p≤0.01), which is paralleled with Sirt-1 and RANKL expression. Regular release of 20µg/kg/h FK866 decreased osteoclasts recruitment (46% decrease, p≤0,005) and alveolar bone surface in resorption (30% decrease, p≤0,05) in in vivo model. Conclusion: Nampt seems to control osteoblasts differentiation in phenotype implicated in osteoclast recruitment.Perspective:In vivo : To study effects of nampt inhibition with FK866 on Sirt-1 and RANKL expression's profile during bone remodeling cycle in the model of alveolar bone remodeling. In vitro : To study the role of nampt on osteoblasts induced osteoclasts differentiation and activity (conditionned media from OB treated with recombinant nampt or OB transfected with nampt siRNA).

Mots-clés

nampt, osteoblasts, bone remodeling, cell differentitation

Phosphate transporters, functional implication in dental mineralization - P42

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Résumé

The importance of phosphate in tooth mineralization suggests a key role for membrane proteins controlling Pi uptake into dental cells. However, contrary to calcium, there is no study allowing to assess the functional implication of the different phosphate transporters in tooth mineralization. As a first step to determine the functional involvement of the 6 known phosphate transporters (Npt1, Npt2a, Npt2b, Npt2c, PiT1 and PiT2) during tooth development and mineralization, we have compared their spatiotemporal expression in mouse tooth germs. Our results obtained by qPCR are in accordance with those obtained by in situ hybridization and immunohistochemistry: PiT1, PiT2 and Npt2b are the phosphate transporters expressed during tooth development. The expression of these 3 transporters increases after birth until about post-natal day 10. At this point, we observed Npt2b & PiT1 predominantly in ameloblasts, and PiT2 in dental pulp. These results are in line with some published results. It remains to be determined whether these proteins act as transporters of phosphate in the tooth. Indeed, considering the pattern of expression (late after the initiation of mineralization) the functional implication of these transporters and their implication in odontogenesis defects remains to be determined. To this aim, we have set up functional models of 2 mineralizing dental cell lines (ALC and M2H4) as well as an organotypic dental germ culture to be investigated with lentiviral shRNA gene silencing techniques. Furthermore, we are currently analyzing dental phenotypes of PiT2 KO mice that interestingly exhibit dental abnormalities.

Mots-clés

Phosphate transporters, odontogenesis, biomineralization

Human Amniotic Membrane for osteoregeneration of calvaria defects - P44

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Résumé

INTRODUCTION: human Amniotic Membrane (hAM) may be an alternative to induced membrane in the case of segmental bone defects. This membrane, composed of an epithelial cell (AEC) layer and of a mesenchymal stromal cell layer (AMSC), has a strong osteoinductive potential. Cryopreserved hAMs are already used for eye cornea and skin burn repair as dressings. Osteoinductive properties of hAM remain to be precisely described. The project analyzes i) the consequences of cryopreservation and ii) the role of epithelial and mesenchymal layers, on the bone regeneration efficiency in a model of calvarial critical defect. MATERIALS AND METHODS:in vitro, fresh and cryopreserved hAM fragments were incubated in a culture medium during one week; metabolic activity and live/dead aspects were analyzed. In vivo, bone defects (3.3 mm diameter) were performed in OF1 adult mice calvaria. Defects remained free or were covered by an hAM fragment, either fresh or cryopreserved in each orientation (AEC or AMSC, n = 8-10 in each group). Sacrifice was performed after 8 weeks and bone regeneration was quantified by X-rays (faxitron and micro-CT) and histological staining. RESULTS: the metabolic activity (Alamar blue assay) was stable in fresh hAM but significantly decreased after cryopreservation ($p < 0.5$). However, a few cells remained alive after thawing and after 7 days, dividing cells were detected (AECs and AMSCs). Eight weeks after surgery and hAM addition to the defects, some mineral focal regions were detected in the presence of cryopreserved hAM as compared with the free defects, but integral regeneration was not detectable. Histology confirmed bone neoformation in these defects. CONCLUSION: the viability of hAM cells was altered due to cryopreservation. However, a partial bone regeneration was observed with cryopreserved hAM.

Mots-clés

human Amniotic Membrane ; bone regeneration; cryopreservation; osteoinduction

Dentin repair in sost ko mice: following of dentin reactions in a model of pulp exposure - P25

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Résumé

Introduction: Sclerostin is a secreted glycoprotein specifically produced by osteocytes. In human, it is encoded by the Sost gene located on chromosome 17. Sost inhibiting mutations have been associated with rare genetic diseases namely Sclerosteosis, Van Buchem disease and Autosomal Dominant Craniodiaphyseal Dysplasia in which patients mainly display a dramatically increased skull thickness. Sclerostin has been described as an antagonist of the Wnt-BMPs signalling pathway and is a strong inhibitor of bone growth. However, its role in the dental structures is still unclear. Objectives: This study aims to describe dentin repair in Sost KO mice and is based on a model of pulpal exposure for which the dentin bridge formation kinetic and the tertiary dentin apposition physiology are well known in WT mice. Materiel and methods: 15 Sost KO mice and 9 WT mice were operated under anaesthesia. Pulp exposure was performed with a diamond bur and a K-file. The pulp was then covered by Mineral Triaggregate Cement (MTA™), a bonded resin composite allowing the tooth structure reconstruction. Mice were followed by micro CT (Quantum FXÒ de Perkin Elmer) at pre- and post D+ 2, 30, 49 and 100 surgery time. At each time, some mice were sacrificed for immunochemistry analysis. Results: No differences were observed between KO and WT mice regarding the pulp volume compared to pre-surgery imagery. At day 30 and at day 49, a faint dentinal bridge was seen for WT mice, whereas nothing (day 30) or a tiny (day 49) dentinal bridge was observed in KO mice. At day 100, KO mice presented a pulp volume clearly decreased compared to WT mice. In some KO mice, dentin obliteration was visible throughout the whole root canal under pulp injury. Masson trichrome colorations confirmed imagery data, but did not reveal any disturbance in tertiary dentin apposition in KO. Immunochemistry with anti-DSP and anti-OPN showed a normal staining distribution in KO tertiary dentin. Conclusion: Sost KO mice presented a tertiary dentin apposition that may be delayed on early stages but enhanced and fastened compared with WT mice after D100. Nevertheless, apart from this kinetic difference, no major difference in immunohistological analysis was observed at this stage.

Mots-clés

Sost, Tertiary dentin, Pulpe, Micro-CT

IMPROVEMENTS IN THE PRODUCTION OF NANOCRYSTALLINE HYDROXYAPATITE - P26

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Résumé

Hydroxyapatite (HAP) is a substance that is most actively used in orthopedia and dentistry as a biocoating of implants in order to improve its osteointegration with bone tissue. In this work Nano-size Hydroxyapatite powders were prepared by the wet chemical precipitation method with calcium hydroxide and orthophosphoric acid solution as calcium and phosphorus precursors, respectively with Ca/P molar ratios of initial reagents is equal to 2.5 it is not a neutralization reaction. The use of ca/p molar ratio at 2.5 means that, the ph of the reaction mixture is self buffered at high pH between 10 and 12, without the addition of any base such as ammonium hydroxide NH4OH as the reported in the literature. This has significant benefits both environmentally and industrially as the synthesis does not require removal of harmful ammonia vapors. Filtered cakes were dried at 90°C and calcined at 300, 500, 700, 900 and 1000°C. The dried and calcined powders were characterized using X-ray diffractrometry (XRD) and Fourier transform infrared spectroscopy (FTIR). Phase evolution characteristics of the powders were studied via X-ray diffractometry as a function of temperature in the range of 90°-1000°C. The indexing, lattice parameter and space group runs on the samples were performed by the Fullprof program. All XRD patterns shows diffraction lines characteristic of hydroxyapatite, confirmed by comparing data obtained with the ICDD - PDF2 card: 00-009-0432. The crystallite size and the crystallinity degree increased from 18 to 56 nm and 35% to 94%, respectively, with increasing of heat treatment temperatures from 90°C to 1000°C.The X ray diffraction analysis reveal the presence of insignificant amounts of calcium oxide as secondary phase identified in the powder treated at 1000°C. Rietveld analysis also revealed high purity and the dimensions of the unit cell of calcined HA, indicating a hexagonal structure. The IR spectrum confirms the formation of hydroxyapatite and suggest the presence of CO32- in hydroxyapatite structure. The Transmission electron microscopy (TEM) micrograph of HA calcined at 900°C reveals that the sample present a rod-like morphology with sizes about 30-50 nm in the short axis and 70-180 nm in the long axis. It can also be seen that the calcined powder exhibited high tendency to agglomerate.

Mots-clés

hydroxyapatite, chemical precipitation, X-ray diffraction, Fullprof program, FTIR spectroscopy.

Up-regulation of FGFR3 signaling in mesenchymal lineages impairs fracture repair in adult mice - P28

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Résumé

Fracture repair is a true regenerative process, which depends on a controlled inflammatory response, followed by the recruitment of skeletal stem cells, deposition of cartilage and bone matrix and skeletal tissue remodeling. When bone repair is compromised by extensive injuries or pathological conditions, surgical intervention may include the use of autografts, allografts, bone graft substitutes and BMPs. The BMP signaling pathway acts in coordination with many other signaling pathways including Wnt, Fibroblast Growth Factor (FGF), Indian Hedgehog and Parathyroid Hormone/Parathyroid Hormone related Peptide. FGFs are involved in various biological functions including skeletogenesis, by influencing cellular proliferation and differentiation. FGFs act via their receptors FGFR1, 2, 3 and 4, the first three being expressed in cartilage and bone cells. Activating mutations in fgfr1, 2 and 3 genes are responsible for craniosynostosis, and mutations in the fgfr3 gene are also associated with chondrodysplasias. The role of FGF signaling during skeletal repair is less well known, but it is clear that the FGF pathway is reactivated during repair [1].Here we used a mouse model that was developed to study the in vivo effects of the activating mutation in fgfr3 (fgfr3Y367C/+). This mutation causes a severe form of dwarfism as observed in the human pathology [2, 3]. Mice expressing the activating fgfr367C/+ mutation ubiquitously die at 8 weeks of age. Therefore, we crossed the fgfr367C/+ mice with the Prx1-Cre mouse line to induce the mutation specifically in the limb mesenchymal lineages during development [4]. These mesenchymal lineages give rise to the skeletal progenitors contributing to fracture repair in the adult. We observed that adult Prx1-fgfr367C/+ mice exhibit a 65% reduction in long bone length ($n=5$; $p<0.001$), which is consistent with the known dwarfism phenotype. We induced non-stabilized tibial fractures in three-month old mice and analyzed the stages of fracture repair via histomorphometry. We observed a severe delay in bone repair as indicated by a marked reduction in callus size, cartilage and bone volumes within the callus of Prx1- fgfr3Y367C/+ mice compared to controls ($n=5$, $p<0.05$). Safranin-O staining at days 7 and 14 post-fracture showed decreased cartilage deposition, delayed and disturbed cartilage hypertrophy in Prx1-fgfr3Y367C/+ mice, indicating a delay in endochondral ossification. Trichrome staining at day 14 post fracture showed reduced bone deposition but accelerated maturation of bone trabeculae. These results show that fgfr3 plays an important role during bone repair and that fgfr3 signaling may be targeted therapeutically to enhance bone repair.

Mots-clés

fracture repair, mouse model, fgfr3

Reinforcements of Si-HPMC hydrogel for cartilage engineering - P29

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Résumé

Cartilage is an avascular and acellular tissue which is not able to heal spontaneously. Because of the increase of lifetime, osteo-articular disorders are a serious public health issue. In this context, tissue engineering has been subject of many studies over the last few years. This strategy is based on the association of cellular therapy with a biomaterial which is able to support cell growth and differentiation. In this context, our lab studies the association of cells with an injectable biomaterial such as silanised hydroxypropylmethyl cellulose (Si-HPMC) hydrogel. The results for cartilage regeneration have shown encouraging. Unfortunately, hydrogels are fragile in comparison to native tissues. Interestingly, it has been shown that cell fate is sensitive to matrix stiffness (Engler et al., 2006). In this context, the aim of our work was to reinforce Si-HPMC by adding nano-particles of silica (laponites).The self cross-linkable polymer (Si-HPMC) was prepared as described in our lab (Bourges et al. , 2002). Hydrogels and reinforced hydrogels were physico chemically characterized to determine their elastic moduli using MARS rheometer (G'), Dynamic Mechanical Analysis (E'). Biological investigations have been done with human nasal chondrocytes (hNC) by determining the cell viability using MTS assay and with Live&Dead kit (confocal analysis) for 7 days in vitro. Finally, the in vivo ability of reinforced hydrogels to support hNC-mediated chondrogenesis has been studied by implantation in the subcutis of nude mice for 6 weeks. Explants have been characterized by histological analysis (Alcian blue, Masson's Trichrome, immunodetection of collagens).Mechanical analysis showed that nanoparticles can reinforce Si-HPMC hydrogel, especially with 1% wt/v laponites which induced a 3 fold increase in the elastic modulus. Laponites used in this study showed no toxicity in vitro. In addition, reinforced hydrogels were shown to support hNC-mediated chondrogenesis in vivo. Cartilaginous nodules containing glycosaminoglycan-rich matrix with type II collagen and aggrecan were evidenced. Indeed, cartilaginous clusters observed in explants were positively stained by Alcian blue and Masson's Trichrome suggesting the production of an extracellular matrix containing glycosaminoglycan and collagen respectively. Our study demonstrates the feasibility of strengthening the Si-HPMC hydrogel while keeping it biocompatible. The next step will be to evaluate the bio-efficacy of reinforced hydrogel to repair cartilage in articular site.

Mots-clés

hydrogel, tissue engineering, cartilage

GPR40 activation by high fat diet decreased bone loss in ovariectomized mouse model - P30

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Résumé

Purpose: GPR40, a free fatty acid receptor, has been shown both in vivo and in vitro to prevent from bone loss by mainly targeting osteoclast resorption activity with a synthetic agonist GW9508. Here, we questioned whether stimulation of the GPR40 receptor by high-fat diet enriched with long chain fatty acids, natural ligands of GPR40, may parallel with its described beneficial effects on bone. Methods: In this study, 9 week-old sham-operated and ovariectomized C57/BL6 wild-type and GPR40-/- mice were fed with control or ANC high-fat diets for 5 weeks. Bone mineral density, body composition, weigh, inflammation and serum circulating bone remodeling parameters were monitored. Results: Although ANC high-fat diet induced a decrease of BMD in sham-operated wild-type, under ovariectomy conditions, mice fed with ANC high-fat diet have a significant higher BMD than GPR40-/- mice. This effect seems to be due to a modulation of the osteoblast/osteoclast coupling by stimulation of GPR40 by fatty acids. Conclusions: In this study, wedemonstrated for the first time that GPR40 limits bone loss induced by ovariectomy upon high fat diet. Taken together; our results demonstrate that GPR40 could mediate beneficial effects of fatty acids mainly by targeting the bone cell coupling and subsequent osteoclastic bone resorption.

Mots-clés

GPR40, fatty acids, ovariectomy, bone loss.

Pentosidine and degree of mineralization are increased in bone from fractured-patients with type 1 diabetes mellitus - P31

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Résumé

Type 1 diabetes (T1D) is associated with increased fracture risk, not explained by the measurement of bone mineral density (BMD). If T1D causes deterioration in “bone quality” rather than reduction in BMD (bone mass) is an important question and little investigated in human. The aim of this study was to analyze the bone matrix (organic and mineral) of iliac bone biopsies, from T1D patients with fragility fracture (FX, n=5), sex and aged-matched T1D patients without fracture (non-FX, n=5), and to compare them to controls (CTL, n=5). All the analyses were performed separately on cortical (cort) and trabecular (trab) bone. Data were then correlated with patient’ information (Weight, Body mass index, duration of diabetes), and Laboratory data (HbA1c, 25(OH)D, creatinine, IGF-1). Non-enzymatic cross-links (pentosidine, PEN), and enzymatic cross-links (PYD and DPD) were examined by HPLC after extraction from embedded bone slices. Degree of mineralization (DMB) was assessed by quantitative microradiography, microhardness was measured by microindentation and bone material properties were obtained by FTIRM. We confirmed that trabecular bone from FX-T1D patients contained significantly higher levels of PEN than in CTL (p=0.04). In bone from non-FX T1D, PEN was not significantly increased but tended to be higher than CTL. PYD was not modified in either T1D group compared to CTL. DPD was decreased in non-FX, only in cortical bone. In trabecular bone from FX-T1D, DMB was higher compared to both CTL (p=0.04) and non-FX T1D (p=0.04). Microhardness tended to increase in non-FX and FX T1D, both in cortical and trabecular bone. Mineral maturity, crystallinity and carbonation were not modified, confirming the absence of an effect on bone remodeling activity. Interestingly, we found significantly positive correlations between HbA1c and PENtrab, HbA1c and DMBtotal, PENtrab and DMBtotal (Fig.). Correlations with DMB were confirmed by mineralization index measured by FTIRM. This suggested that in T1D, HbA1c can predict both accumulation in bone of pentosidine and lead to a higher DMB. Indeed, it appears that bone from FX-T1D is more mineralized than both non-FX T1D and CTL. In conclusion, we showed that high serum HbA1c impacts both organic and mineral matrix in bone biopsies. Increase in both Pen and DMB were observed in bone from FX-T1D. Association of both high PEN and degree of mineralization could stiffen bone matrix and lead to fractures. Figure: Spearman correlations between HbA1c, pentosidine and degree of mineralization

Mots-clés

Diabetes Type 1 - AGEs - Bone Mineral - Bone Quality -

ADAPTATION OF CULTURES OF DENTAL PULP PROGENITORS TO CLINICAL REQUIREMENT - P32

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Résumé

Introduction: Human dental pulp cells (HDPC) are generally isolated and cultured with xenogeneic products and in stress conditions that may alter their biological features. However, guidelines from the American Food and Drug Administration and the European Medicines Agency currently recommend the use of protocols compliant with medicinal manufacturing. Our aim was to design an ex vivo procedure to produce high amounts of HDPC for dentin/pulp and bone engineering according to these international recommendations. Methods: HDPC isolation was performed from pulp explants cultures. After appropriate serum-free medium selection, cultured HDPC were immunophenotyped with flow cytometry. Samples were then cryopreserved for 510 days. Post-thaw cell doubling time was determined up to passage 4 (P4). Karyotyping was performed by G-band analysis. Osteo-odontoblastic differentiation capability was determined after culture in a differentiation medium by gene expression analysis of osteo-odontoblast markers and mineralization quantification. Results: Immunophenotyping of cultured HDPC revealed a mesenchymal profile of the cells, some of which also expressing the stem/progenitor cell markers CD271, Stro-1, CD146 or MSCA-1. Post-thaw cell doubling times were stable and similar to fresh HDPC. Cells displayed no karyotype abnormality. Alkalin phosphatase (ALPL), Osteocalcin (OCN) and Dentin sialophosphoprotein (DSPP) gene expression and mineralization were increased in post-thaw HDPC cultures performed in the differentiation medium compared to cultures in control medium. Conclusions: We successfully isolated, cryopreserved, amplified and differentiated human dental pulp progenitor cells with a medicinal manufacturing approach. These findings may constitute a basis on which to investigate how HDPC production can be optimized for human pulp/dentin and bone tissue engineering.

Mots-clés

Human dental pulp, tissue engineering, immunophenotyping, MSCA-1, cryopreservation, osteo-odontoblast differentiation

Fabrication and characterization of chitosan_'hyaluronic acid porous scaffold with cell colonization - P33

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Résumé

Strategies for bone tissue engineering and regeneration rely on bioactive scaffolds to mimic the natural extracellular matrix (ECM) and act as templates onto which cells attach, proliferate and synthetize ECM compounds.1 Bone scaffolds need to be biocompatible, biodegradable, with structural and mechanical features close to bone tissue.2In recent work, we developed a porous three-dimensional (3D) hybrid chitosan (CHI)/hyaluronic acid (HA) scaffold suitable for tissue engineering applications. Rheological characterization highlighted sol-gel transition at 37°C after 10 min of incubation. The hydrogels were freeze-dried to make 3D porous scaffolds. Structural, physicochemical and mechanical characterizations were investigated. Microscopical observations showed that the scaffold provide a porous interconnected network. Chemical crosslinking by adding genipin3 was required for stabilization, and reinforcement of the structure, keeping them in stable form for a long term. Uniaxial compression measurement indicated a significant 100 times increase in Young's modulus through this chemical crosslinking. Biocompatibility of the scaffold was finally studied through LDH and DNA quantification assay after perinatal stem cell seeding. LDH assay showed that scaffold has no cytotoxic effect on stem cell survival. However, no significant evolution in DNA quantification over time was seen. Cells imaging showed the presence of round cells inside the scaffold.We have developed a biocompatible CHI/HA porous scaffold for bone tissue engineering. Our next step will be to investigate the stem cells commitment into osteoblastic lineage.

Mots-clés

bone tissue engineering, 3D, chitosan, hyaluronic acid, stem cells

DENTAL PULP REGENERATION IN MINI-PIG - P34

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Résumé

Teeth are particularly exposed to caries, trauma or malformations, in which the tissue loss often requires difficult reconstructions and causes esthetic and functional disabilities. These lesions strongly affect the patient's quality of life. Up to now, the therapy for deep dental pulp lesions has mostly been oriented towards endodontic treatment, which consists in pulp removal, mechanical enlargement of the root canal and placement in the cleaned pulp cavity of a bio-inert material sealed with root canal cement. However, post-operative evaluation of such treatments reveals a high amount of failures resulting in high tooth morbidity and potential induction of systemic bacterial infection. Based on the presence of cells endowed with stem cell properties in the tooth pulp (Gronthos et al. 2000; Huang 2009), we propose an alternative to the classical endodontic treatment, by substituting the injured pulp with an engineered pulp, to maintain tooth nutrition and sensitivity, therefore decreasing tooth morbidity (Sun et al. 2011).The aim of this project is to validate the feasibility of pulp regeneration in a preclinical model: mini-pig. This large animal model offers similarities with human, in terms of diphyodonty, dental development, eruption, size, anatomy and diet (Weaver et al. 1962).The proposed methodology consists in the partial substitution of the mini-pig pulpal tissue, by an engineered pulp, consisting of dental pulp stem cells preconditioned or not by hypoxia and seeded in a collagen scaffold. For that purpose, two teeth non-essential for mastication were extracted to isolate dental pulp stem cells. These cells were expanded and placed in 3D collagen matrix. In a second surgical step, molar pulp tissue was removed, featuring the pulp pathology, and replaced by the artificial pulp.The function of the reconstructed pulp tissue was monitored over time by CT angiography. After the sacrifice of the animal, analysis of the regenerated tissue was carried out by CT imaging, histology and molecular biology approaches. A special emphasis was given to the angiogenesis in the reconstructed pulp tissue and in the regeneration of sensitive fibers.The use of a large animal model exceeds the limitations of the rat model used so far, particularly in terms of scale, chewing function and anatomy.This project would therefore constitute a preclinical stage for innovative treatments for dental lesions, considering the availability of dental pulp stem cells (loss of primary teeth, third molar or second premolar extraction for orthodontic treatment plan).

Mots-clés

dental pulp regeneration, dental pulp stem cells, pulp injury

RUNX2 AND VDR EXPRESSION IN THE CONTROL OF OSTEOBLAST FUNCTION AND BONE FORMATION - P35

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Résumé

Runx2 is a member of the runt family of transcription factors whose expression is essential for cell commitment to the osteoblast lineage but is also necessary for osteoblast precursors to differentiate, and to mature osteoblasts to be activated. Interestingly, intriguing in vivo and ex vivo data indicate that transgenic cells overexpressing Runx2 under the control of the 2.3kb rat Col1a1 promoter are blocked at an early stage of differentiation, with impacts on bone resorption and remodeling rate. Our goal was to understand how Runx2 overexpression affects osteoblast differentiation, and to identify the downstream effectors of Runx2, by transcriptome analysis. The aim of this project was to identify in vivo new genes responsible for or at least involved in the aging-related bone loss observed in mice overexpressing Runx2.Analysis of the transcriptome led to the identification of 2,028 genes with altered expression in long bones, depending on mouse age (1, 4 and 8 months of age) and genotype (Runx2 TG vs. WT mice). A series of reference genes and of markers of osteoblast that were formerly identified as Runx2 target genes, as well as the most promising impacted genes were validated by Q-PCR. This validation allowed us to make a selection of genes regulated by Runx2 in order to perform a functional study. Since several genes are vitamin D regulated genes, we will focus here on the regulation of vitamin D receptor (VDR) expression by Runx2.VDR is highly expressed in tissues involved in calcium homeostasis. In bone, VDR is expressed in osteoblast and mediates the effect of vitamin D on calcium mobilization from bone. Transcription factors controlling the activity of the VDR promoter in bone are largely unknown. We identified the presence in the mouse VDR gene of 4 binding sites (OSE2) for the Runx2 factor, one in the 1st intron, 3 in the close promoter region. Co-transfection of a Runx2/Cbfa1 expression vector enhanced the activity of the mouse VDR promoter in reporter vector experiments. EMSA and competition experiments suggested the binding of Runx2 on several of the predicted OSE2 sites, specific mutations abolishing these bindings. Superhift experiments using specific antibodies confirmed the binding of Runx2 on several OSE2 sites. Functional analysis of the mouse VDR promoter, by mutating one or several OSE2 sites, revealed that two OSE2 sites (-315 and -156) are classical positive OSE2 sites while another one (-211) represents a negative OSE2 site.

Mots-clés

Runx2, VDR, bone formation

BONE DISEASE IN CYSTIC FIBROSIS : IMPAIRMENT OF MATURATION KEY FACTORS EXPRESSION IN OSTEOBLASTS AND POTENTIAL THERAPEUTIC - P36

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Résumé

Cystic fibrosis (CF) is a lethal genetic disorder caused by mutations in the CFTR gene. Low bone mineral density is frequently observed in patients with CF, even in youth people [1]. This was evidenced to be multifactorial (nutritional deficiencies, chronic infections and inflammation, delayed puberty, frequent use of glucocorticoid drugs) but the direct effect of CFTR mutation in osteoblastogenesis, i.e on the bone formation, remains poorly studied. We investigated whether the F508del-CFTR mutation, the most common mutation observed in CF patients, could affect the expression of osteoblast genes relative to differentiation (COL1A1, BGLAP,SPARC, ALPL, RUNX2, SP7) and maturation (BMP2, SMAD1/2, OPG, RANKL, COX-2) in human osteoblasts.F508del osteoblasts were obtained from trabecular bone explants prepared from three CF patients homozygous for the F508del-CFTR mutation and a CF patient with the F508del/G542X mutation in CFTR. Normal osteoblasts used as control, were obtained from trabecular bone explants of adults who underwent trauma surgery. Chloride conductance of CFTR channel was evaluated by iodide (I-) efflux in primary normal osteoblasts and was greatly reduced in F508del osteoblasts. Compared to healthy osteoblasts, real time PCR data evidenced no significant difference in osteoblasts gene expression related to differentiation in F508del osteoblasts. By contrast, a decreased expression of genes relative to osteoblastic maturation was found in the four CF patients-derived osteoblasts. In fact, F508del bone cells exhibited a diminished expression of SMAD2, COX-2 and a higher RANKL/OPG mRNA ratio. Furthermore, we found that the basal production of both OPG protein and COX-2 metabolite prostaglandin E2 (PGE2), two well-recognized osteogenic activators, was reduced by F508del osteoblasts [2]. Treatment with a CFTR corrector compound (VRT-534 also known as C18 provided by Vertex Pharmaceuticals) markedly increased the F508del-CFTR chloride activity and ameliorated both the RANKL/OPG mRNA ratio and COX-2/PGE2 expression and production in F508del osteoblasts [3]. Of importance, COX-2 activity and PGE2 have been previously identified as key mediators in the initial step of osteogenesis and the latter step of osteoblast maturation, supporting the role for COX-2 and PGE2 in the regulation of skeletal growth [4]. Considering the presence of CFTR in the osteoblasts and the fact that the pathophysiology of the bone disease is linked to absent bone formation, we hypothesize that the CFTR corrector C18 might be a good candidate to propose as therapeutic agent to fight against bone disease in CF patients.

Mots-clés

Cystic Fibrosis ; Bone disease ; human primary osteoblasts ; F508del–CFTR mutation ; COX2/PGE2 ; RANKL/OPG

Potential of dental pulp stem cells for bone defect regeneration - P37

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Résumé

Background: Dental pulp stem cells (DPSC), originating from the neural crest, display a capacity of differentiation towards various mesenchymal fates, including odontoblasts and osteoblasts, and have the ability to restore human mandible bone defects (d uino et al 2009), enhance mineralization (Coyac et al 2013) and promote angiogenesis (Souron et al 2014). We aim to study and evaluate the use of DPSC as a therapeutic agent to restore damaged orofacial bones. Material and methods: Bilateral parietal circular defects (5mm diameter, agreement CEEP2.JLS.174.10) were drilled in calvaria Wistar rats and filled with dense rat-tail polymerizing type I collagen 3D hydrogel containing (test) rat DSPC (4.106 cells per mL of collagen) or not (control). Radiographical investigation by in vivo micro-tomography, histological and histomorphometrical analysis were performed each week during 5 weeks. Results:In vivo, radiological dynamic examinations and quantification of bone regeneration volume and density were gradually and significantly increased (respectively x3 and x5 at 5 weeks) ($p<0,01$) in the implanted area when matrices were seeded with DPSC. Microarchitecture analysis of the regenerated area revealed a well organized cortical and trabecular bony material with a significantly increased trabecular number (x2 at 5 weeks) ($p<0,01$) in the regenerated bone when matrices were seeded with DPSC. Histologically, regenerated bone was alkaline phosphate and TRAP positive, indicating restored bone forming and resorbing activities within implanted collagen matrices. Conclusion: This work shows that dense collagen 3D matrices are efficient for bone regeneration, and identifies DPSC as excellent candidates for bone tissue engineering to heal craniofacial bone defects.

Mots-clés

Dental Pulp Stem Cells, bone, tissue engineering

Down-regulation of Sirtuin type 1 (Sirt 1) expression in bone marrow of anorexia nervosa mouse model: potential involvement in osteoporotic phenotype - P38

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Résumé

Background: Osteoblasts and adipocytes share a common mesenchymal stem cell origin. Therefore, it has been suggested that the accumulation of marrow adipocytes observed in bone loss is caused by a shift in the commitment of mesenchymal stem cells from the osteogenic pathway to the adipogenic pathway.The sirtuin family, specifically sirtuin type 1 (sirt 1) are implicated in the regulation of osteoblast and adipocye differentiation. Indeed, reduced sirt1 protein level accompanied by increased marrow adipogenesis was found in the bone marrow of ovarectomized mice (ref). In vitro, sirt1 activation by Resveratrol was shown to increase osteoblastogenesis and reduce marrow adipogenesis (1-3). More recently, sirt1 was described as an important regulator of bone mass and a repressor of sost gene encoding for sclerostin, a bone formation inhibitor (4).Despite the recently described role of sirt1 in bone biology and osteoporosis and despite its known increased expression in soft tissues of fasted animals, no study focused on the link between sirt1 and osteoporosis related to Anorexia Nervosa. A mouse model mimicking numerous consequences of severe Anorexia Nervosa and based on separation associated with time restricted feeding (SBA protocol) (5) was used to obtain mice with significant bone alterations. In addition, a co-differentiation medium allowing both osteoblast and adipocyte differentiation of mouse bone marrow stromal cells was validated (6). Thus the aim of this work is to determine if sirt1 alterations could be linked to bone loss observed in Anorexia Nervosa. Methods: Female C57BL6 mice of 8 weeks of age have been submitted to SBA protocol for 7-8 weeks. Femurs and tibias were removed and cleaned of connective tissue. Gene expression of sirt 1 was determined on bone marrow stromal cells (BMSCs) after 48h of culture. To define the effect of sirt 1 on osteoblastic and adipogenic differentiation, bone marrow stromal cells (BMSCs) from SBA and control mice were harvested from the half of bones. Theses cells were treated or not by Resveratrol or Sirtinol, an activator and an inhibitor of sirt1 activity respectively, and differentiated into osteoblasts and adipocytes in the co-differentiation medium for 14 days. Gene expression of sirt1 was measured by real-time PCR and the effect of sirt1 on osteo/adipogenesis was studied by biochemical analyses (Oil Red O, Ca/protein) and by measuring the mRNA expression of some adipocyte and osteoblast markers. The second half of bones were used for organotypic culture: bones were treated or not with regulator of sirt1 activity (resveratrol and sirtinol) for 10 days, in order to determine the effect of sirt1 on bone microarchitecture and adiposity. Results: After 48 h of adhesion, BMSCs of SBA mice presented a decrease of 80% in mRNA expression of sirt1 versus BMSCs from control (CT) mice. Interestingly, in BMSCs from SBA mice, mRNA expression of adipocyte markers (PPAR-gamma 2, Leptin, Adiponectin and Glut4) was increased whereas mRNA osteoblast marker expression (Runx2, ALP, Osterix and osteocalcin) was decreased. Microarchitecture of organotypic cultures is now been determined. Conclusion and Perspective: This data demonstrate for the first time that the know increase in sirt 1 expression in various soft tissues of food restricted mice is accompanied by a decrease of this expression in BMSCs. The results also suggest that this sirt 1 decrease is associated with a rise of adipogenesis and a decrease in osteoblastogenesis and could be implicated in bone loss observed in SBA mice. This study will be completed by adiposity analysis of organotypic culture.

Mots-clés

Sirt1, BMSCs, Co-differentiation medium, Adipogenesis, Osteoblastogenesis

Molecular investigations of bone quality from osteoporotic women treated with Alendronate or Strontium Ranelate after 12 months - P39

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Résumé

Introduction:
In osteoporosis, the bone remodeling is disrupted which leads to the alteration of bone quality. The administration of anabolic or anticatabolic agents is commonly used to cure osteoporosis. The Alendronate (ALN) inhibits bone resorption. The Strontium Ranelate (SrRan) has a dual effect on bone formation and bone resorption. Their clinical benefit is recognized but their mechanism at molecular level needs to be improved. The aim of this work is to compare the effect of SrRan and ALN on bone quality at molecular level.

Material and Methods:
Iliac biopsies were done on osteoporotic women at baseline (M0, n=10) and after 12 months (M12, n=10) for each treatment (ALN or SrRan). Back Scattered Electron images (BSE) were acquired to locate new bone and old bone. Elemental images (Sr, Ca, and P) were acquired to locate Sr incorporated in bone. Microspectroscopy Raman was performed on new and old bone over all biopsies. Physico-chemical parameters (PCP) were calculated from the ratio of specific bands on Raman spectra: mineralization (1450/960), type-B carbonatation (1070/960), HPO4/PO4 (1004/960), hydroxyproline/proline (871/854), crosslinks (1680/1670), PG/AmideIII (area ratio 1362–1408/1216-1304) and crystallinity (1/Full-Width-at-Half-Maximum 960 cm-1) [1]. The results are interpreted as function of the treatment, the nature of bone and the tissue age.

Results:
The mineralization, the type-B carbonatation and the crystallinity increase significantly in old bone compared to new bone, independently of the treatment and the duration therapy (M0 or M12) as expected in literature [2].
In new bone, the ALN-M12 is characterized by an increase of the mineralization (+9.3%, p=0.0026) in trabecular bone and PG/Amide III (+8.5%, p=0.0030) compared to the baseline ALN-M0.
In new bone, the SrRan-M12 is characterized by a decrease of the type-B carbonatation (-9.6%, p<0.0001), of the crystallinity (-1.8%, p=0.017) and of the PG/AmideIII (-8.3%, p=0.0047) compared to the baseline SrRan-M0.
In old bone, no difference according to the treatment was observed between M0 and M12.

Conclusion:
Both molecules modify the composition of bone but their mechanism of interaction on mineral seems different. The suppression of bone remodeling by ALN, which increase the bone lifespan, leads to an enhancement of the secondary mineralization. This effect may explain the increase of the mineralization. A substitution mechanism may occur in mineral during Sr intake which could explain the release of carbonate and the decrease of crystallinity. In addition, ALN and SrRan seem to have an opposite effect on the mineralization mechanism of collagen matrix according to PG/AmideIII results.

Mots-clés

Raman, Strontium, Alendronate, Mineralization, Collagen

ASSOCIATION D'UN LYSAT DE MOELLE OSSEUSE ET DE BIOMATÉRIAU PHOSPHOCALCIQUE DANS LA RECONSTRUCTION DE L'OS IRRADIÉ - P40

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Résumé

L'ostéoradionécrose mandibulaire est une complication sévère de la radiothérapie dans le traitement des carcinomes des voies aéro-digestives supérieures. En alternative à sa prise en charge par lambeau osseux vascularisé micro-anastomosé, l'ingénierie tissulaire se développe sur le plan préclinique. L'utilisation chez l'animal de phosphates de calcium biphasés (BCP) associés à de la moelle osseuse totale (MOT) permet d'obtenir une ostéoformation significative dans l'os irradié (1,2,3). Cette réparation tissulaire est induite par la MOT grâce à plusieurs mécanismes, dont le mécanisme paracrine. Le lysat de MOT étudie ce mécanisme en ne conservant que les facteurs solubles tels que les facteurs de croissance et les cytokines (4). Donnant des résultats significatifs dans la réparation de divers tissus (4,5,6,7,8), le lysat de MOT n'a pourtant pas été évalué dans la reconstruction de l'os irradié. L'objectif de cette étude est donc d'étudier l'intérêt du lysat de MOT, en injections in situ ou intraveineuses, dans la reconstruction de l'os irradié, par rapport à l'association de référence BCP-MOT. Vingt rats ont été irradiés au niveau des membres postérieurs par une dose unique de 80 Grays. Trois semaines plus tard était réalisée la chirurgie avec création de défauts osseux de taille critique. Le groupe « intra-osseux » (n=12) permettait l'étude de l'utilisation du lysat in situ, avec 6 conditions d'implantation (défaut vide, BCP, MOT, BCP-MOT, lysat seul, BCP-lysat). Le groupe « intraveineux » (n=8) recevait, après implantation selon 4 conditions (défaut vide, BCP, MOT, BCP-MOT), 4 injections intraveineuses de lysat de MOT pendant 2 semaines. A 5 semaines des implantations, les échantillons ont été explantés pour analyse qualitative en histologie et quantitative en microscopie électronique à balayage. Une étude de l'immunogénicité du lysat a été conduite, par réactions mixtes lymphocytaires (MLR).Les résultats ont montré une néoformation osseuse significativement plus importante pour le groupe « intraveineux » par rapport au groupe « intra-osseux ». Bien que l'association BCP-MOT reste la plus efficace en utilisation in situ, les injections IV de lysat de MOT ont entraîné un bénéfice plus important, en présence de MOT, avec ou sans biomatériau (Figure 1). Histologiquement, une moelle osseuse richement cellularisée au niveau des défauts osseux était observée après injection intraveineuse, et non lors d'une utilisation locale du lysat. Les MLR ne retrouvaient pas de prolifération après 3, 5 et 7 jours d'incubation d'un lysat d'une espèce avec des lymphocytes d'une autre espèce.Notre étude a évalué l'intérêt du lysat de MOT dans la reconstruction de l'os irradié. Nous avons obtenu des résultats significatifs en faveur des injections intraveineuses de lysat de MOT. De nouvelles perspectives sont ouvertes, mais il reste à confirmer ces résultats et à comprendre les mécanismes d'action du lysat.

Mots-clés

Radiotherapy ; Bone tissue ; Biomaterials ; Tissue Engineering ; Paracrine Communication

Pannexin 1 and pannexin 3 regulate osteoblastic differentiation of human bone marrow mesenchymal stem cells within a three-dimensional macroporous scaffold - P41

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Résumé

Pannexins (Panx) are vertebrate channel proteins identified by their similarity to connexins, the gap junction proteins. The pannexin family consists of three members, Panx1, Panx2, and Panx3. The roles of Panx as large-pore ion and metabolite channels are recognized in many physiological and pathophysiological scenarios but the role of these proteins in cellular processes of human stem cells remain unknown.In this study we investigated the localization and expression of Panx1 and Panx3 in human bone marrow mesenchymal stem cells (HBMSCs) seeded in 2D or within a polysaccharide-based 3D matrix, composed of pullulan and dextran, which promotes multicellular interactions and mimics an appropriate microenvironment for stem cells. We show that culturing HBMSCs, without osteogenic factor, within the 3D scaffolds promotes Panx1 and Panx3 expression and revealed the role of Panx1 in the compaction of the 3D cellular aggregates. This role was demonstrated by the use of two Panx1 specific inhibitors, Probenecid and the mimetic peptide 10panx1. In 2D conditions, the inhibition of Panx1 lead to a reduced expression of osteogenic markers by HBMSCs. However, no significant effects were attained in 3D cultures, suggesting specific roles of Panx1 in the osteogenesis process of HBMSCs. Also, we could observe a correlation between the gene expression profile of Panx3 and those of osteoblastic markers (i.e. ALP, OCN, CaSR, Cbfa1). During time of culture, we also observed this correlation with expression profile of connexin43 (Cx43), a component of GAP junction and with the P2X7 receptor of ATP, within the 3D macroporous matrix.To conclude, this study reveals new roles of pannexins in the osteogenic differentiation of multipotent HBMSCs, with the comparison between 2D and 3D cultures within a macroporous polysaccharide-based matrix.

Mots-clés

Human bone marrow mesenchymal stem cell; Pannexin 1; Pannexin 3; Osteogenesis; 3D microenvironment.

Facteurs environnementaux et morphogenèse du thèque chez les diatomées - P45

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Résumé

Les diatomées sont des microalgues qui contribuent de façon importante aux cycles biogéochimiques de la silice et du carbone essentiellement dans les environnements marins et plus particulièrement dans les zones costières. Même si la formation de leur squelette est dépendante de la disponibilité en silice dissoute dans ces environnements, il a été montré que certains facteurs biotiques et abiotiques peuvent influencer la morphologie et les ornementations de celui-ci. Cependant peu d'information existe sur les altérations du processus de morphogenèse sous contraintes environnementales et en particulier sur la vitesse d'apparition et l'étendue des variations morphométriques des frustules. La capacité d'acclimatation et d'adaptation des diatomées reste aussi une question importante. Afin de comprendre les mécanismes d'acclimatation et d'analyser des différences interspécifiques éventuelles nous avons exposé une espèce de diatomée dite côtière et une plutôt océanique à des gradients de pH et de salinité. Les analyses démontrent que même si l'incorporation du silicium est peu affectée, les variations de la morphologie du thèque sont dépendantes de la durée de l'exposition et de la nature du facteur étudié. Il semble que le processus de biominéralisation est robuste au regarde de l'impact de ces mêmes gradients de pH et de la salinité sur la réponse génique chez ces espèces.

Mots-clés

Acclimatation, Morphogenèse, Microalgues, Facteurs environnementaux

Imagerie in vivo de la vascularisation par micro-scanner - P46

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Résumé

Depuis plusieurs années, les techniques d'imagerie in vivo sont devenues des outils indispensables pour la recherche préclinique ainsi que pour la recherche clinique. Parmi ces techniques, la micro-tomographie par rayons X permet l'acquisition rapide d'images structurales dépendamment de l'atténuation des rayons X par les tissus. Si cette technique est particulièrement adaptée à la visualisation des tissus minéralisés, l'observation de tissus mous ou de la vascularisation peut néanmoins être réalisée par l'injection de produit de contraste. Notre objectif a été de mettre en évidence la vascularisation tissulaire par utilisation d'un produit de contraste non létale et une méthode rapide et reproductible d'injection. Après anesthésie par voie gazeuse, les souris C57BL/6J ont reçues une injection intravasculaire (rétro-orbitaire) de 150 µl d'ExiTron nano 12000 (Viscover, Miltenyi Biotec), puis ont été imagées par micro-tomographie ("Quantum FX Caliper, Life Sciences, Perkin Elmer, USA).Un seuillage des vaisseaux a permis de les mettre en évidence parmi les tissus mous (cœur, foie, rate, rein…) ainsi que les espaces intra-osseux : cavités médullaires des fémurs et des tibias, encéphale, au niveau mandibulaire, omoplates… Cette méthodologie non létale a permis la mise en évidence des réseaux vasculaires extra et intra-osseux et autorise un suivi in vivo et longitudinal des processus de vascularisation et d'angiogenèse. Ce travail est supporté par la FRM (DGE20111123012)

Mots-clés

Micro-CT, in vivo, vascularisation,

Defective skeletal mineralisation in PiT2/Slc20a2-deficient mice - P47

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Résumé

Skeletal mineralisation is a process of fundamental importance to all vertebrate animal species. During skeletal growth and remodelling, calcium and phosphate (Pi) are required for the formation of biological apatites. The rate at which mineralisation occurs is dependent, in part, on the local availability of Pi and calcium. Although implication of Pi transporters in mineralisation process appears evident, the identity of this (or these) protein(s) is yet to be determined. In vertebrae, the type III cotransporters (PiT1 and PiT2) are the only NaPi cotransporters identified so far in skeletal tissues, and are thus considered as essential suppliers of Pi for skeletal mineralisation, despite the absence of in vivo evidence. This putative role in bone mineralization was also derived from in vitro studies that have showed that PiT1, but not PiT2, expression is regulated by factors regulating bone cells. However, we recently demonstrated that PiT1 hypomorphic adult mice have a normal bone mineralisation.To assess the role of PiT2 in bone development, growth and mineralisation, we underwent the phenotypic characterisation of PiT2 knockout mice (French Ethical approval n° 02286.01). We show that PiT2-/- mice are subviable and that 50% of the PiT2-/-mice are dying off around birth. After birth, we observe that PiT2-/- mice are growth retarded and exhibit impaired skeletal mineralisation. Quantitative faxitron analyses show lower bone mineral content in PiT2-/- mice compared with controls. In addition, PiT2-/- bones are weaker and less stiff than controls. Analyses of histological sections of the upper tibia from PiT2-/- mice show a decreased bone formation and also a reduced growth-plate mineralisation at postnatal day 16.We are now investigating expression of key regulators of Pi homeostasis. To this aim, we are performing conventional biochemical analyses and RT-qPCR from bone, kidney, gut and blood samples.Altogether, these data suggest that PiT2 is a key sodium-phosphate cotransporter for skeletal mineralisation.

Mots-clés

mineralisation, sodium-phosphate cotransporter, phosphate homeostasis, low bone mineral content

FLUORIDE IMPACTS IRON METABOLISM CAUSING A WEAKENED AND DECOLORED TOOTH - P48

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Résumé

Fluoride is commonly used to increase enamel mineralization. However, dental fluorosis could occur in case of excessive fluoride intake during tooth mineralization. The resulting hypomineralization and discoloration depend at least on the fluoride dose and the genetic background. The pigmentation of rodent tooth is caused by an iron-bearing compound that is assumed to be incorporated in vivo into the enamel by ameloblasts. The aim of the present study is to analyze the relationship between fluoride excessive intake and iron metabolism in ameloblasts in order to explain the fluoride adverse impact on enamel color and hardness. We used a mouse model deleted for one allele of the heavy chain ferritin (Hft +/-), a key component of iron storage in cells, to highlight the targets impacted by chronic fluoride administration. Four groups of mice were constituted: HFt +/+ and HFt +/- treated or not with 5 mM NaF. Iron accumulation was evidenced by Perl's staining and SIMS imaging to detect with higher sensitivity and specificity the Fe element in a sub-cellular level. Expression of iron metabolism components was analyzed by immunohistochemistry and quantitative RT-qPCR. First, fluoride reduced iron accumulation in ameloblasts (whatever the type of analyze). It also decreased expression of HFt as well as of key enamel genes, KLK4 (main enamel protease), amelogenin and enamelin. On the other hand, it increased ferroportin expression which is the iron transporter responsible for iron release. Second, the lack of HFt allele was associated to decreased HFt and ferroportin expression and increased amelogenin and enamelin. Interestingly, incisors of HFt +/- mice were less colored than those of wild types. Concerning the combination of NaF and HFt invalidation: We found a repression of DMT-1 expression which is one of the component that permits the entry of iron into the enamel organ cells. In other words, NaF prevented the entrance of iron into ameloblasts explaining the loss of enamel pigmentation. In addition, HFt expression was strongly reduced and ferroportin up-regulated, thus decreasing even more iron accumulation. Interestingly, incisors of HFt +/- NaF-treated mice were white and broken. In conclusion, the relationship between fluoride and iron metabolism is here reported for the first time. Our results demonstrate the functional involvement of iron accumulation in enamel coloration and quality. In addition, fluoride effects observed in human and rodents can be explained, at least in part, by its role on expression of key component of iron metabolism.

Mots-clés

Fluoride; Iron; Ameloblasts; HFt +/- mice

ROLE OF BONE MARROW ADIPOCYTES IN THE ALTERED BONE REMODELING OF THE OVARIECTOMY MODEL - P49

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Résumé

Introduction & aim: In osteoporosis, the BMD loss is paralleled by a severe increase in bone marrow adiposity, which suggests a detrimental impact of these adipocytes in bone homeostasis. Indeed, several in vitro studies have shown that adipocyte secretions (adipokines) can alter the differentiation, function and survival of osteoblasts or osteoclasts. However, primary mature Bone Marrow Adipocytes (BMA) remain poorly studied and their capacity to release these factors and to interfere with bone remodeling has not been clearly established in vivo.Our main aim is to characterize the functional phenotype of BMA with regard with their secretory capacity in the ovariectomy model. Methods: Fourteen-week-old C57BL/6 mice are sham-operated or ovariectomized and analyzed after 4 or 14 weeks following the surgery. Trabecular architecture and bone marrow adiposity are measured in the proximal tibia using µ-CT and histomorphometry respectively. Femur and tibia BMA are separated from the other bone marrow cells, while visceral adipocytes are isolated from the perigonadal fat pad. Gene expression analysis of the different adipocytes is then performed using real-time PCR.Results: The µCT measurements confirm the progressive loss of trabecular bone in the proximal tibia of ovariectomized mice compared to sham mice. As the trabecular bone volume declines between 4 and 14 weeks following ovariectomy, the percentage of adiposity increases in the tibia metaphysis. Moreover, an enlargement of the adipocyte diameter is observed which suggests BMA metabolism changes between the two time points. After 14 wks of ovariectomy, isolated BMA have decreased expression levels of transcriptional factors involved in adipogenesis and classical adipokines compared to visceral adipocytes. However, some MMPs and RANKL mRNAs are well-expressed in BMA. Moreover, three Wnt-signaling inhibitors (sFRP4, sFRP1 and DKK1) are found highly expressed in BMA compared to visceral adipocytes and the other bone marrow cells. A preliminary comparison of the BMA after 4 and 14 weeks following ovariectomy shows that the expression level of sFRP4 is lower at the earliest time point whereas adiponectin mRNA level is similar. Conclusions: The gene expression analysis supports that BMA exhibit an altered differentiation state compared to that of the peripheral adipocytes. Moreover, in the ovariectomy-induced bone loss, BMA are characterized by the expression of several factors known to interfere with osteoblastogenesis and osteoclastogenesis. The comparative analysis at two time points suggests that the BMA phenotype evolves as the bone loss worsens. Altogether, our data support that BMA can contribute to the bone loss in a model of postmenopausal osteoporosis.

Mots-clés

osteoporosis, bone marrow adipocyte, ovariectomy

Blocking TGF-beta signaling pathway inhibits the development of osteosarcoma lung metastases - P50

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Résumé

Osteosarcoma is the main malignant primary bone tumor in children and adolescents for whom the prognosis remains poor, especially when metastases are present at diagnosis (survival rate drops to 20% when lung metastases were detected). Because TGF-beta has been shown to promote metastases in many solid tumors, we investigated the effects of inhibition of the TGF-beta/Smad cascade on osteosarcoma behavior. To this end, two independent procedures, a pharmacological approach with TGF-beta Receptor I inhibitor (SD-208) and a molecular approach using the natural Smad inhibitor (Smad7), was tested. We first demonstrated that TGF-beta levels are higher in the serum of osteosarcoma patients compared to healthy volunteers. We also showed that Smad7 slows the growth of the primary tumor and increases mice survival. In this context, we demonstrated that Smad7 expression does not affect osteosarcoma cell proliferation but affects the microarchitectural parameters of bone. In addition, Smad7-osteosarcoma bone tumors expressed lower levels of osteolytic factor RANKL, suggesting that Smad7 overexpression affects the vicious cycle established between tumor cells and bone cells by its ability to decrease osteoclast activity. Interestingly, we finaly showed that Smad7 overexpression in osteosarcoma cells and SD-208 inhibits the development of lung metastasis. In this context, we demonstrated that Smad7 and SD-208 reduced the capacity of osteosarcoma cells to invade Matrigel in Boyden migration chambers and gelatin zymography identified reduced MMP-2 secretion by osteosarcoma cells. These results suggest that the inhibition of TGF-beta/Smad signaling pathway could be a promising therapeutic strategy against the tumor progression of osteosarcoma specifically against the development of lung metastases

Mots-clés

osteosarcoma, TGFbeta, Lung metastases

Mesenchymal stem cell differentiation towards osteoblast using 3D culture models - P51

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Résumé

Introduction. The antitumor potential of new molecules is tested first on tumor cell lines cultured on plastic (2D) and in different animal models. However, less than 10% of the molecules validated by preclinical tests are effective in clinical tests. To reduce this failure rate, an intermediate stage of testing molecules could be achieved in vitro 3D culture systems including the stroma on which cancer cells develop. In order to test new molecules targeting bone tumor cells, we develop 3D scaffold presenting some characteristics of the bone environment. Methods. Mesenchymal stem cells (MSCs) derived from human bone marrow, were cultured either on a biphasic calcium phosphate ceramic (BCP) or nanofibers of polycaprolactone in osteogenic medium (100 nM dexamethasone, 250 µM ascorbic acid, 10 mM β-glycerophosphate). Cell viability studies, electron microscopy, immunohistochemistry (alkaline phosphatase, type I collagen and bone sialo protein), quantification of mineralization (alizarin red) and collagen (Sirius Red) and studies gene expression (COL1A1, CBFA1, ALP, SOST, BSP, BMP2, OC) were performed. Results. Viability of MSCs was observed on long culture times (30-90 days) and their differentiation to the osteoblast phenotype was confirmed on both 3D culture systems. The abundant presence of type I collagen and the presence of non-collagenous bone proteins were observed, indicating the synthesis of an osteoid extracellular matrix. Conclusion / Discussion. These culture systems have not resulted in a lamellar bone tissue in vitro, but an osteoid matrix was obtained and used to culture osteosarcoma or Ewing_sarcoma cells.

Mots-clés

Mesenchymal stem cells, 3D culture, osteoblasts

TRABECULAR BONE ARCHITECTURE – ITS PIVOTAL ROLE IN BONE FRAGILITY - P52

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Résumé

Introduction: During growth, trabeculae emerging from the growth plate thicken. Peripherally placed trabeculae coalesce forming metaphyseal compact-appearing cortical bone (CC) while centrally placed trabeculae form cancellous bone with a transitional zone (TZ) between. Deficits in trabecular number or reduced thickening may delay corticalization leaving an enlarged and porous TZ. In adulthood, a negative bone balance and accelerated intracortical remodeling cavitates cortex. We hypothesized that these processes result in a higher porosity in women with forearm fractures across life. Methods: We quantified microarchitecture using high resolution-peripheral quantitative computed tomography (HR-pQCT, isotropic voxel size 82 micron) at the non-dominant or non-fractured distal forearm in cases with, and age-matched controls without, distal radius fractures with a ratio 1:2. After exclusions due to movement artefacts, we analysed: i) 110 girls (12±3 yrs, range:7-18), ii) 100 pre-menopausal women (28±6 yrs, range:18-44) (1) and iii) 164 post-menopausal women (65±8 yrs, range:51-89). Bone compartment cross sectional areas (CSA), trabecular architecture and porosity of the, TZ and total cortex (TC) were assessed using StrAx1.0 (2) and expressed as a function of the total CSA to control for bone size. Results: Girls with fractures had a 3% smaller CC-CSA and a 3% reciprocally higher TZ-CSA that were 12% and 4% more porous than controls respectively (all $p \leq 0.03$). Trabecular BV/TV was 21% lower (8% fewer, 4% thicker, all $p<0.05$). Pre-menopausal cases had normal CC- and TZ-CSA but TZ porosity was 2% higher and trabecular BV/TV was 30% lower (7% fewer, 3% thinner) (all $p<0.05$). Post-menopausal cases had normal CC- or TZ-CSA that were respectively 25% and 5% more porous and 39% lower BV/TV (37% fewer, 10% thicker) (all $p<0.0001$). By multivariate regression analyses, CC and TZ porosity was associated with fracture independent of trabecular vBMD in girls. In pre-menopausal women, trabecular vBMD was associated with fracture independent of porosity. In post-menopausal women CC and TZ porosity was associated with fracture independent of trabecular vBMD.Inferences: At all ages, cases had higher cortical porosity and deficits in trabecular number. Metaphyseal fragility is the result of the incomplete corticalization of fewer trabeculae emerging from the growth plate early in life leading to higher peak porosity producing more surfaces for unbalanced and rapid remodelling after menopause.

Mots-clés

Bone growth, Bone metaphyses, fragility fractures

Liste des présentations orales "classiques" concourant pour un prix de communication

		Session	N°	Graphisme /10	Clarté de l'exposé /10	Contenu scientifique /10	Questions /10
Muscle injury impairs bone regeneration in adult mice	Julien Anais	1	O1				
PiT1 mediates survival of chondrocytes from endoplasmic reticulum-induced stress in vivo	Greig Couasnay	1	O2				
miR-199a/b-5p and Runx2 collaborate to regulate Wnt/β-catenin pathway	Mylène Zarka	1	O3				
IIB OR NOT IIB: DEVELOPMENT OF AN ORIGINAL SCREENING METHOD BY FLOW CYTOMETRY TO CHARACTERIZE HUMAN MESENCHYMAL STEM CELLS...	Hugo Fabre	1	O4				
MINERALIZATION OF THE MOUSE TRACHEA IS A SUDDEN AND EARLY PHYSIOLOGICAL EVENT	Chaohua DENG	2	O6				
FGF23 REGULATES MMP13 THROUGH FGFR1 IN HUMAN OA CHONDROCYTES VIA PI-3K AND ERK PATHWAYS	Mathilde GUIBERT	2	O7				
IN VIVO MEASUREMENT OF PH AT THE SITES OF CALCIFICATION OF THE RED CORAL CORALLIUM RUBRUM	LE GOFF Carine	2	O8				
Osteoclast activity regulates hematopoietic stem cell niches during inflammatory bowel disease	Agathe BOUCOIRAN	3	O10				
HIF signaling in skeletal progenitors promotes breast cancer growth and metastasis through systemic production of CXCL12	Claire-Sophie Devignes	4	O13				
CALPAIN-6 EXPRESSION IDENTIFIES A STEM CELL POPULATION IN OSTEOSARCOMA	Caroline Andrique	4	O15				
miR-146a deficiency in Ly6Chigh monocytes contributes to pathogenic bone loss during inflammatory arthritis	Meryem AMMARI	4	O16				

Liste des posters doctorants concourant pour un prix de communication

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INVESTIGATION OF THE CALCIUM-SENSING RECEPTOR EXPRESSION IN MONOCYTES ISOLATED FROM SYNOVIAL FLUIDS	Alice Séjourné	P1				
HYPOXIA-DEPENDENT DNA METHYLATION TO CONTROL THE CHONDROCYTE PHENOTYPE	Anne-Laure Durand	P2				
A NEW ALTERNATIVE IN BONE REGENERATION: COMBINATION OF CHITOSAN/HYDROXYAPATITE SCAFFOLD, NACRE ACTIVE COMPOUNDS AND STEM CELLS	Anne-Sophie WILLEMIN	P3				
HYDROLYZED COLLAGEN PROMOTES OSTEOBLASTOGENESIS AND PRESERVES BONE MASS IN OVARIECTOMIZED MICE	audrey daneault	P4				
THE ESTABLISHMENT OF MICROFLUIDIC CO-CULTURE SYSTEM TO STUDY THE INTERPLAY BETWEEN SENSORIAL NERVOUS SYSTEM AND MESENCHYMAL STEM CELLS IN VIEW OF OSTEOGENESIS	BRUNO PAIVA DOS SANTOS	P5				
Bioingeneering of mandibulare reconstruction in cancer surgery	Camille EHRET	P6				
The flavonoid fisetin promotes osteoblasts differentiation through Runx2 transcriptional activity	Cédric Darie	P7				
OSTEOCLASTS ACTIVATE CHONDROCYTE CATABOLISM THROUGH S1P PRODUCTION.	CHAHRAZAD CHERIFI	P8				
SILICON IN VEGETABLES: IN VITRO BIOACCESSIBILITY, BIOAVAILABILITY AND BIOLOGICAL ACTIVITY IN DIFFERENT TARGET TISSUES (INTESTINE AND BONE)	D'Imperio Massimiliano	P9				
EFFECT OF THE LEUCINE RICH AMELOGENIN PEPTIDE ON ENAMEL STRUCTURE AND MINERALIZATION	Elvire Le Norcy	P10				
SEPARATION AND IDENTIFICATION OF THE OSTEOGENIC COMPOUNDS OF NACRE USING ION-EXCHANGE RESIN ASSOCIATED WITH A MINERALIZATION CELL MODEL	Ganggang ZHANG	P11				
Bone loss in early phases of rat arthritis is predictive to disease severity	Guillaume COURBON	P12				
A new therapeutic approach for achondroplasia and hypochondroplasia: Tyrosine Kinase Inhibitor BN016	KOMLA-EBRI Davide	P13				
DEVELOPPEMENT D'UN MODELE D'OSTEOGENESE IN VITRO AU SEIN DE MATERIAUX 3D	Laura JUIGNET	P14				

DISTINCT EXPRESSION OF IL-36 AND THEIR ANTAGONISTS IN RHEUMATOID ARTHRITIS	Marie-Astrid Boutet	P15				
CYSTIC FIBROSIS BONE DISEASE: AN ELEVATED RANK-L/OPG PROTEIN RATIO IN OSTEOBLAST WITH THE F508DEL-CFTR MUTATION	Martial Delion	P16				
Glycosyl-Nucleosyl-Fluorinated - Collagen injectable hydrogel for tissue engineering: A new scaffold for bone regeneration.	Mathieu Maisani	P17				
Un vol spatial d'un mois à bord du bio satellite russe BION M1 fragilise sévèrement le squelette des souris	Maude Gerbaix	P18				
INSIGHT INTO THE EXTRACELLULAR PHOSPHATE SENSING MECHANISM, THE KEY STEP FOR AN APPROPRIATE FGF23 SECRETION	Nina Bon	P19				
GENERATION OF NUCLEUS PULPOSUS PROGENITOR CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS.	Pauline Colombier	P20				
Osteoblastic syndecan-2 is a new orchestrator of Wnt signaling in bone cells	RAFIK MANSOURI	P21				
A NEW ES CELL LINE AS AN ALTERNATIVE SOURCE FOR NEURAL CREST DERIVED STRUCTURES IN TISSUE ENGINEERING.	Soledad ACUNA MENDOZA	P22				
A SYSTEMATIC REVIEW ON PRE-CLINICAL AND CLINICAL APPLICATIONS OF RAPID PROTOTYPING IN BONE TISSUE ENGINEERING	Vera Guduric	P23				
SHORT-TERM EFFECTS OF ZOLEDRONIC ACID ON BONE QUALITY AT MOLECULAR LEVEL IN THE RAT MANDIBLES	Xavier COUTEL	P24				
NAMPT IS REQUIRED FOR OSTEOBLASTS ACTIVITY AND OSTEOCLASTOGENESIS IN BONE REMODELING	Bassam HASSAN	P27				
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