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A WNT CANON ORCHESTRATING SKELETAL DEVELOPMENT

Hartmann C[1], Hill TP[1], Später D[1], Birchmeier W[2], Taketo MM[3]
Institute of Molecular Pathology (lMP), Vienna, Austria[1], Max-Delbrück Center for Molecular Medicine, Berlin, Germany[2], Department of Pharmacology Graduate School of Medicine, Kyoto, Japan[3]

Osteoblasts and chondrocytes are involved in building up the vertebrate skeleton and are thought to differentiate from a common mesenchymal precursor, the osteo-chondroprogenitor. Although numerous transcription factors involved in chondrocyte and osteoblast differentiation have been identified, little is known about the signals controlling lineage decisions of the two cell types. A detailed in vivo and in vitro conditional loss- and gain-of function analyses revealed that b-catenin activity is necessary and sufficient to repress the differentiation of mesenchymal cells into Runx2 and Sox9 positive skeletal precursors [1]. This suggests that b-catenin levels have to be low in order for mesenchymal cells to enter the skeletal lineage. Furthermore, by conditionally deleting b-catenin in the limb and head mesenchyme we and others showed that b-catenin is required for osteoblast lineage differentiation [1-2]. Osteoblast precursors lacking b-catenin are blocked in differentiation and develop into chondrocytes instead. In vitro experiments demonstrate that this is a cell-autonomous function of b-catenin in an osteoblast precursor. The synovial joints of the appendicular skeleton are probably also originating from a skeletal precursor common to all three lineages, chondrocytes, osteoblast and joint interzone [3]. Studies of the conditional loss-of function b-catenin limbs and of mice lacking some of the Wnt-ligands expressed in the joint region suggest that the canonical Wnt-signaling is required in the joint regions to actively repress the differentiation of chondrocytes. Thus allowing proper formation of the joint and maintaining joint integrity at later stages. In conclusion, our studies show that canonical Wnt/b-catenin signaling is essential during skeletal lineage differentiation, preventing trans-differentiation of osteoblastic cells, joint interzone cells and synoviocytes into chondrocytes.

References:
1 Hill TP, Später D., Taketo MM, Birchmeier W, Hartmann C (2005) Dev. Cell 8, 727-738
2 Day TF, Guo X, Garret-Beal L, Yang Y (2005) Dev. Cell 8, 739-750
3 Akiyama Ddagger H, Ddagger JE, Nakashima K, Balmes G, Iwai N, Geng JM, Zhang Z, Martin JF, Behringer RR, Nakamura T, de Crombrugghe B (2005) PNAS 102, 14665-14670

Keywords: canonical Wnt-signaling, osteoblasts, chondrocytes, mouse skeleton

REGULATION OF BONE REMODELLING

Väänänen K
Department of Biomedicine, University of Turku, Finland

Bone undergoes continuos remodelling via coodinated action of various cell types. Although major cellular activities needed for bone remodeling are now rather well established, namely bone resorption and bone formation, there are still several open questions concerning e.g. initiation of remodeling cycle and recruitment of osteogenic as well osteoclastic cells. Molecular mechanisms used by osteoclasts to resorb mineralized bone involve secretion of acid and proteinases into resorption lacunae and continuous removal of degradation products. This is related to an extensive membrane trafficking in osteoclasts.

In addition to the mechanism of actual resorption also regulation of the differentiation of osteoclasts is understood in some details. However, mechanisms behind the initiation of remodelling cycle remains a mystery although a role for osteocytes has been suggested. Since osteocytes are most abundant cells in bone and they are ideally distributed inside the bone matrix it has been suggested that they may have a central role in the regulation of bone remodelling.

Local bone resorption by osteoclasts initiates remodeling cycle. There is some evidence to suggests that loading induced microfractures may initiate local remodeling event. However, mechanisms how appearance of microfractures is signalled to bone resorbing cells or their precursors are unknown. We have developed methods to isolate osteocytes from cortical bone and calvariae in order to study their cell biology. In addition, new culture models to study osteocyte and osteoclast interactions will also be discussed.

LATE MOLAR AND BONE PHENOTYPE IN HOMOZYGOUS MSX 2 KI MICE

Berdal A[1], Aioub M, Descroix V, Robert B*, Lézot F, Néfussi JR
INSERM U714, Universities Paris 6 and Paris 7; Pasteur Institute*; Paris, France

Msx (Muscle segment homeobox) genes control epithelial-mesenchymal interactions leading early tooth development. The present study aims to analyse the potential impact of Msx 2 in mouse mandible and tooth from 7 post-natal days to 6 months in a knock-in mouse strain.
Mice from the same litters, produced by mating heterozygous Msx 2 +/- mice, were analysed after PCR-genotyping. Microradiography, and histochemical (morphology, amelogenin and ameloblastin) studies were performed on Msx2 +/+, Msx2 +/- and Msx2 -/- mice.

Amelogenesis imperfecta and dentin dysplasia were presently recorded, suggesting the existence of a similar phenotype in homozygous knock-in (here) and previously studied knock-out Msx2 -/- models. In addition, growth and homeostasis of alveolar bone appeared affected, with an irregular and partial root ankylosis. A proximo-distal gradient in dental and periododontal alteration grades was observed. The first, second and third molars systematically showed a distinct phenotype, particularly evident in dentin and bone. Since Msx 2 invalidation affects ameloblast survival and Msx 2 acts as an inhibitor for amelogenin gene transcription in vitro, enamel proteins were studied in crown and root epithelium. Effectively, an up-regulation of amelogenin and also ameloblastin proteins appeared to involve giant Malassez epithelial rests of Msx 2 -/-, a phenomenon presumably instrumental on adjoining bone cells.

These data support the major role of Msx 2 in tooth and extend its function post-natally and to the associated bone physiology. The affected molecular cascades within the physiological complex formed by tooth and alveolar bone, which might involve enamel proteins, have to be delineated. The observed Msx2 -/- phenotype gradient suggests the existence of interfering/redundant factors on this Msx 2 signaling pathway, variable depending on the molar-type (Msx1 and its endogenous antisense RNA ?, see I. Fernandes's joint presentation).

Keywords: bone, tooth, Msx2

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BONE MARROW CELLS CAN DIFFERENTIATE INTO AMELOBLAST- AND ODONTOBLAST- LIKE CELLS WITHOUT CELL FUSION

Hu B[1,2,3], Unda F[4], Bopp-Kuchler S[1,2], Jimenez L[4], Wang XJ[3], Wang SL[3], Lesot H[1,2]
INSERM U595[1], Faculty of Dentistry, Louis Pasteur University, France[2], Faculty of Stomatology, Capital University of Medical Sciences, PR China[3], Department of Cell Biology and Histology, Faculty of Medicine and Dentistry, University of the Basque Country, Spain[4]

Ameloblasts disappear during tooth eruption while enamel can be damaged due to caries, genetic diseases or injury. Except for cultured enamel organ cell, no cell resources have yet been found to replace or regenerate ameloblasts. Thus searching for non-dental cell resource, who may differentiate into ameloblasts, together with finding conditions to do so remain a big challenge. Bone marrow cells (BMC) have already been shown to be able to give rise to dental mesenchymal cells (Ohazama et al., 2004). Furthermore, recent reports suggested that bone BMC can also give rise to different types of epithelial cells (for review see Krause, 2005). In this study, attempts were thus made to test the hypothesis that BMC might also serve as resource for ameloblasts. EGFP BMC mixed with dental epithelial cells were engrafted in the newly formed enamel organ of reassociations cultured in vitro (Hu et al., 2005; Hu et al., in press). After 20 days culture, when located at the epithelial-mesenchymal junction, in the inner dental epithelium, BMC no longer incorporated BrdU, elongated, polarized, expressed amelogenin and ameloblastin genes and secreted amelogenin and MMP20. Interestingly, BMC could also be found in the odontoblast layer. In this location they expressed DSP/DPP gene and secreted DSP and DMP-1 in a polarized manner. In this system, the phenotype of BMC was determined by their position. Two distinct mechanisms have been proposed in order to explain how bone marrow cells can give rise to different cells types: cell fusion or direct differentiation. By using FISH method to identify X and Y chromosomes, we proved that the differentiation of BMC into either ameloblast- or odontoblast-like cells did not involve in cell fusion. These results from the present study show for the first time that BMC have the ability to simultaneously differentiate into ameloblast- and odontoblast-like cells. This offers new possibilities for ameloblast and odontoblast regeneration as well as whole tooth tissue engineering.

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References: Hu, B., Nadiri, A., Bopp-Kuchler, S., Perrin-Schmitt, F. & Lesot, H. (2005) J. Dent. Res. 84, 521-525; Hu, B., Nadiri, A., Bopp-Kuchler, S., Perrin-Schmitt, F., Peters, H. & Lesot, H. (2006) Tissue Eng. In Press; Krause, D. S. (2005) Ann. N. Y. Acad. Sci. 1044, 117-124; Ohazama, A., Modino, S. A., Miletich, I. & Sharpe, P. T. (2004) J. Dent. Res. 83, 518-522

Keywords: tooth, tissue engineering, ameloblasts

PERICYTES OF DENTAL PULP: A NEW MODEL TO STUDY REPARATIVE DENTINOGENESIS

Alliot-Licht B, Bluteau G, Lopez-Cazaux S and Guicheux J
INSERM U791, Osteoarticular and Dental Tissue Engineering, School of Dental Surgery, Nantes, France

Preserving tooth vitality plays a crucial role in the maintenance of dentition. The effects of aggressive injuries (i.e.: trauma, caries,..) may induce pulp exposure that in turn leads to corresponding odontoblast death and bleeding. The successful outcome for reparative dentin is clearly dependent on the type and location of injury, the age of the tooth, the treatment modality and bacterial invasion. The odontoblasts of second generation, also called odontoblast-like cells secrete reparative dentin. Although knowledge on some active molecules present in the dentinal tissue supplies new information on regeneration and repair in the dentin-pulp complex, the key question regarding the identity of the progenitor cells giving rise to odontoblasts-like is not currently understood. Because it is hypothesis that perivascular cells could be the progenitors of odontoblast-like cells, we developed a model in which pericytes from dental pulp tissues are isolated and cultured. This new approach focuses on spontaneous and induced differentiation pathways of the pericytes from dental pulp tissue in order to better understand their potential role in the dental reparative process.

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Keywords: pericytes, reparative dentinogenesis

COEXPRESSION OF RGS5 AND NOTCH3 mRNA IN THE PERICYTE-vSMC AXIS AFTER PULP INJURY

Lovschall H[1], Mitsiadis T[3], Jensen KH[1], Kjeldsen AL[1], Poulsen K[2]
[1]Royal Dental College,
[2]Department of Molecular Biology, Faculty of Health Sciences, University of Aarhus, Denmark
[3]Craniofacial Development, King's College Dental Institute, London, UK

Stem cells from the tooth pulp have been isolated and shown to develop dentin ex vivo, and they are suggested to originate from pericytes (Shi S & Gronthos S, 2003). Rgs5 is one of several pericyte markers (Betzholtz C et al., 2005), and Notch signaling has been suggested to specify arterial development in the vascular system (Claxton S et al., 2004). We previously described activated expression of Notch signaling genes after pulp injury (Lovschall et al., 2005; Mitsiadis 1999). In this study our aim was to explore Rgs5 and Notch3 mRNA expression during development and regeneration of injury in adults. Hoechst was in this study used as background labeling. The mRNA expression was analyzed by in situ hybridization in rat incisors, in developing mice teeth, and in adult first upper rat molars with or without capping with Dycal. Notch3 protein expression was also examined during tooth repair after cavity preparation and cariogenic pulp injury by immuno-histochemistry. The Notch3 immunolabeling was most intense in cells of the new-formed vessels. We observed high perivascular hybridization signals from Rgs5 and Notch3 mRNA in pulp capillaries and central arterioles of developing mice molars and incisors. The expressions increased in molars with pulp injury in comparison to control molars. During early pulp repair the hybridization signals upregulated in vascular pericyte locations occasionally with downregulation close to the trauma. In conclusion, vascular Rgs5 and Notch3 expression increased after pulp injury, and coexpression of Rgs5 and Notch3 was observed corresponding to capillary and arteriolar pericytes, with more extensive Rgs5 expression along the pericyte-vascular smooth muscle cell axis in central pulp arterioles.

FGF AND TGFß1 SIGNALING IN NORMAL AND MUTATED OSTEOPROGENITORS

Ferretti P and Santos-Ruiz L
Developmental Biology Unit, UCL Institute of Child Health, London WC1N 1EH, UK

Single point mutations in fibroblast growth factor receptors (FGFRs) cause premature fusion of cranial sutures (craniosynostosis), which result in abnormal craniofacial morphology. Our aim is to better understand how FGFR mutations affect osteprognitors behaviour and pathways regulating bone differentiation. To this purpose we are using MC3T3 osteoblastic cell lines either untransfected or stably expressing mutated (R2-C278F) or wild type (R2-WT) human FGFR2, and primary cell lines from bone biopsies of syndromic patients. R2-C278F cells display lower proliferative index than control and R2-WT cells, and differentiate prematurely, as indicated both by changes in morphology and expression of bone differentiation markers. This is consistent with the phenotype observed in osteprogenitors derived from syndromic patients. The FGFR inhibitor SU5402 reduces growth less extensively in mutated than control cells, suggesting that in R2-C278F cells there is overall inhibition rather than increased activation of FGF signaling. This is consistent with compensatory up-regulation of endogenous FGFR2 transiently observed in R2-C278F cells. All cell lines can respond to FGF, though significant differences are observed. At high concentrations, FGF2 and FGF18 increase proliferation of both control and R2-WT cells in 1% and in10% serum, though to a lower extent. In contrast, R2- C278F cells respond to FGF only in low serum-containing medium. In order to assess whether treatment with TGFß1, previously shown to increase osteoprogenitor proliferation, can improve survival and growth in mutated cells, we have studied its effect at different serum concentrations. In low serum TGFß1 increases cell number in all cell lines, with R2-WT showing the largest response. This effect is potentiated in 10% serum in control and WT-R2 cells, but not in R2-C278F cells. All together our results suggest that impaired FGF signaling affects the cellular response to TGFß, linking the two signaling pathways in osteoprogenitor development.

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Key words: osteoprogenitor, FGFR, FGF, TGFß

EXTRACELLULAR MATRIX MINERALIZATION IN OSTEOBLAST CULTURES IS DUALLY INHIBITED BY BOTH PYROPHOSPHATE AND ITS UPREGULATION OF OSTEOPONTIN

Addison WN[1], Azari F[1], Kaartinen MT[1,2] and McKee MD[1,3]
[1]Faculty of Dentistry, McGill University, [2]Dept. of Medicine and
[3]Dept. of Anatomy and Cell Biology, Faculty of Medicine, McGill University,
Montreal, QC, Canada

Osteopontin (OPN) is a highly phosphorylated matrix protein that inhibits mineralization by binding to growing crystal surfaces. Our recent data indicates that OPN levels are regulated by another mineralization inhibitor - inorganic pyrophosphate (PPi). The PPi anion consists of two phosphate (Pi) molecules linked by a hydrolyzable ester bond. Its ubiquitous presence in "soft" tissues is thought to prevent them from mineralizing, whereas its degradation in bone and teeth may facilitate mineralization. While the crystal-binding properties of PPi are largely understood, less is known about effects on osteoblasts. We have used MC3T3-E1 osteoblast cultures to investigate OPN gene regulation by PPi. Mineralization in MC3T3-E1 cultures was dose-dependently inhibited by OPN. Dephosphorylation of OPN by tissue-nonspecific alkaline phosphatase (TNAP) significantly reduced its mineral-inhibiting potency, suggesting that OPN inhibits mineralization via its negatively charged Pi residues. Treatment of the cultures with PPi led to elevation of OPN levels and inhibition of mineralization. Analysis of common signaling pathways revealed that PPi induced OPN expression via the ERK1/2 and P38 MAPK kinase pathways. Inhibitor studies showed that PPi induction of OPN does not appear to be Protein Kinase A-, C- or G-dependent. OPN regulation by PPi was also insensitive to Probenecid (an anion transport inhibitor), Foscarnet (a Pi import inhibitor) and Levamisole (a TNAP inhibitor), suggesting that increased OPN levels did not result from changes in Pi. Interestingly, PPi also inhibited mineralization in a dose-dependant manner that was abolished by TNAP hydrolysis. In conclusion, this presents a scenario where PPi, an anionic small-molecule inhibitor of mineralization regulates the secretion of OPN, a proteinacious inhibitor, and that both these inhibitors can be regulated by TNAP hydrolysis to influence biomineralization in skeletal and dental tissues.

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Keywords: Osteopontin, Pyrophosphate, Osteoblast

PARTIAL RESCUE OF DENTAL PHENOTYPE OF VITAMIN D RECEPTOR-ABLATED MICE BY CALCIUM AND PHOSPHATE NORMALIZATION

Descroix V[1], Hotton D[1], Asselin A[1], Kato S[2], Lézot F[1], Berdal A[1]1
[1]Laboratoire de Biologie-Oro-Faciale et Pathologie, INSERM U714-IFR 58, Université Paris 7, Université Paris 6 Institut Biomédical des Cordeliers, Escalier E, 75270 Paris Cedex 06, France
[2]Institute of Molecular and Cellular Biosciences, Tokyo University, 1-1-1 Yayoi, Bunkyo, Tokyo 113-0032, Japan.

The biological activity of the main metabolite of vitamin D, 1a,25 dihydroxyvitamin D3 (1,25(OH)2D3), is mediated by a high-affinity receptor, namelly the vitamin D nuclear receptor (VDR). Until now, more than 30 organs, including teeth, and many cell types, such as dental cells, are known to express VDR. Vitamin D has been demonstrated to be intimately involved in tooth formation. Several proteins implicated in the dental biomineralization process were shown to be sensitive to vitamin D deficiency and 1,25(OH)2D3 administration. Vitamin D bioinactivation, via either VDR invalidation or nutritional depletion, leads to rickets with abnormal dental formation. The purpose of this study was to comparatively analyse dental defects associated to rickets in VDR-/- and vitamin D deficient mice of first and second generation. We focused on (1) the additional effect of calcium and phosphate by comparing hypocalcemic and nutritionally rescued normocalcemic VDR-/- mice, (2) the distinct impact of maternal effect by comparing first and second generations of normocalcemic VDR-/- mice. We thus have undertaken microradiographic, histological, immunohistochemical and molecular analyses in these different mice models of rickets (genetic and nutritional). Interestingly, calcemia normalization in VDR-/- hypocalcemic mice, after weaning, restored the major part of rickets-associated dental defects with the exception of dental cell differentiation. Furthermore, the previously reported alterations for tooth morphogenesis in vitamin D-deficiency was revealed to be related to an additional maternal effect in VDR -/- mice. This study is the first-one which discriminates the respective and synergic impacts of coupled vitamin D/VDR, maternal effect and calcium/phosphate in tooth development and morphogenesis.

Keywords : Vitamin D, Calcium, maternal effect

REGULATION BY GLUCOCORTICOIDS OF CALVARIA DIFFERENTIATION AND MINERALIZATION IN RAT FETUS

Fernandes I, Nadra R, Pibouin L, Berdal A and Menuelle P
Laboratory of Oro-facial Biology and Pathology Inserm U714, University of Paris 7, France

In late gestation, the first centers of ossification in the rat fetus appear concomitantly to the glucocorticoid (GC) secretion peak, physiological regulators of growth and differentiation of many organs. Moreover, transcription factors such as Msx, Dlx, Runx and Osterix (Otx) are key regulators of mineralized tissue differentiation.

Thus, we investigated the possible regulation of these genes by GC in combination with alcaline phosphatase (ALP), a marker of osteoblast early differentiation and osteocalcin (OC), the marker of osteoblast late differentiation, both in vitro, in primary cultures of 17-day-old fetal rat calvaria cells and in vivo, in calvaria from E16 to P1.5. In vivo as well as in vitro, Msx1 sense and antisense, Msx2, Dlx3, Dlx5, Runx2, Osterix, ALP and OC mRNA were detected by RT- real time PCR. In vivo, Msx1 sense and antisense transcripts expression decreased concomitantly to the GC peak whereas Msx2, Dlx3, Dlx5, Otx, Runx2 and ALP transcripts expression increased and OC mRNAs were induced since E18. In vitro, dexamethasone (DEX), in conditions mimicking GC peak in rat late gestation, induced extracellular matrix mineralization associated with increased 45Ca incorporation and ALP activity. Moreover, in vitro, Msx1 sense and antisense transcripts were down-regulated whereas Msx2, Dlx3, Dlx5, Otx, and Runx2 transcripts were up-regulated by physiological concentrations of DEX together with ALP and OC expression according to in vivo results.

Thus in regards to our results and the literature, we can postulate that GC can promote mineralized tissue differentiation and OC expression either by their acute increase of Runx2, Msx2, Dlx3, Dlx5 and Osterix expression and by down-regulation of Msx1 sense and antisense expression.

Key words: Msx1, glucocorticoids, bone.

DYNAMIC EXPRESSION OF HMGB1 DURING TOOTH DEVELOPMENT

Christersson C, Lind T, Ohlsson M-L, Wendel EM and Fried K
Center for Oral Biology, Novum, Karolinska Institutet, Huddinge, Sweden

HMGB1 is a nuclear and cytosolic protein, which is involved in a number of biological processes, and can act as e.g. a transcription factor, a growth factor or a cytokine. To elucidate a possible role for HMGB1 in tooth development, we studied the protein expression of HMGB1 and its receptor RAGE during the late fetal and early postnatal (E20-P10) period of the rat, using immunocytochemistry. HMGB1 expression was low during fetal and newborn stages of tooth development. However, from P5 and on, this was followed by a marked increase in levels of this protein in most dental cell types. Expression was particularly high in ameloblasts and odontoblasts at regions of ongoing mineralization. Although most HMGB1 immunoreactivity was confined to cell nuclei, it was present in both ameloblast and odontoblast cytoplasm as well. From P5 and on, odontoblasts also showed RAGE immunoreactivity. Immunoblots performed on extracts from bovine dentin demonstrated a principal band at ~27 kDa, indicating that HMGB1 might participate in mineralization. However, RT-PCR showed that HMGB1 mRNA levels appeared to remain unchanged in human dental pulp cells in an in vitro model of dentin production, which may suggest that HMGB1 is not involved in at least initial stages of dental mineralization. The expression of both protein and receptor in odontoblasts indicates an autocrine /paracrine mechanism for HMGB1 in these cells.

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Key words: HMGB1, tooth development

TRANSGLUTAMINASE ACTIVITY AND FIBRONECTIN CROSSLINKING IS REQUIRED FOR MATRIX MINERALIZATION AND MC3T3-E1 OSTEOBLAST DIFFERENTIATION

Al-Jallad HF, Nakano Y and Kaartinen MT
Faculty of Dentistry, McGill University, Montreal, QC, Canada

Transglutaminases (TGs) are a group of protein-crosslinking enzymes that have been implicated in the formation of mineralized tissues, particularly mineralized cartilage. TGs create isopeptide bonds between lysine and glutamine residues in their substrate proteins. The TG family consists of 9 genes of which TG2 and FXIIIA are expressed by chondrocytes, but very little is known of the expression and role of TGs in osteoblasts and bone. In this study we analyzed the expression, activity and function of TGs in differentiating osteoblasts. MC3T3-E1 (subclone 14) pre-osteoblast cultures were treated with ascorbic acid and beta-glycerophosphate to induce cell differentiation, matrix production and mineralization. We demonstrate that, like chondrocytes, MC3T3-E1/C14 osteoblasts express two TG isoforms - TG2 and FXIIIA during their differentiation. Abundant TG activity assessed by *in situ* labelling of live cultures and by in vitro biochemical assays was observed during cell differentiation which increased significantly after thrombin treatment, which activates FXIIIA. Ascorbic acid treatment, which stimulates collagen secretion and assembly, also stimulated externalization of TG activity, likely from FXIIIA which was externalized upon this treatment as analyzed by immunofluorescence microscopy. Inhibition of TG activity in the cultures by cystamine resulted in complete abrogation of mineralization attributable to decreased matrix accumulation and an arrested state of osteoblast differentiation as measured by decreased levels of bone sialoprotein, osteocalcin and alkaline phosphatase. TG activity was most critical for mineralization at an early phase of the differentiation program. *In situ* substrate labelling experiments during these early days showed that the sole TG substrate was fibronectin. Immunofluoresence studies showed that TG activity was required for the formation of a fibronectin-collagen type I network during the early stages of matrix formation and assembly. This network, in turn, appeared to be essential for further matrix production and progression of the osteoblast differentiation program, and ultimately for mineralization. Collectively these data show that TG activity and matrix crosslinking are critical for osteoblast differentiation.

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Keywords: osteoblast differentiation, mineralization, transglutaminases

ADAMTS-1 METALLOPROTEINASE ACTIVITY INFLUENCE COLLAGEN TYPE I PROCESSING IN OSTEOBLASTIC CELLS

Karlström E, Rehn A, Christensson C, Wendel M and Lind T
Center for Oral Biology, Karolinska Institutet, Sweden.

The ADAMTS-1 protein (A Disintegrin And Metalloprotease with ThromboSpondin repeats) is a member of a family of secreted multi-domain neutral endopeptidases. It is composed of an N-terminal signal peptide, a metalloprotease domain containing a zinc-binding site, a cysteine-rich region and three putative disintegrin loops. ADAMTS-1 is expressed as a 110-kDa protein, but after processing is present both as a 85-kDa and a 67-kDa protein.

The expression of ADAMTS-1 has been associated with inflammation, ovulation, angiogenesis, and cellular proliferation. Furthermore, more research has led to the conclusion that it probably also has a role in bone remodelling.

Bone, mainly composed of collagen type I and hydroxyapatite mineral, undergoes continuous site-specific degradation that is balanced by new bone synthesis. It has previously been shown that Matrix metalloproteinases (MMP) can degrade and generate collagen fragments, which could recruit, activate or initiate bone resorption. The ADAMTS-1 protease domain is similar to the protease domains of MMP's, which likely gives an overlap of their functions. This suggests that ADAMTS-1 could very well be involved in activities previously regarded as MMP activity.

We recently showed that the ADAMTS-1 protein was located to osteogenic rat cells both in vitro and in vivo using an anti ADAMTS-1 antibody in western blots and immunohistochemistry.
In a new study we show a indication that ADAMTS-1 is associated to collagen type I fibrils in bone through Immuno electron microscopy of mouse femur osteoid and that ADAMTS-1 activity induce release of type I collagen fragments into conditioned media of cultured osteoblastic cells.

Furthermore we show that ADAMTS-1 activity induce fibrillar collagen breakdown and this also indicates a role for ADAMTS-1 in bone formationremodelling.

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Keywords: ADAMTS-1, Collagen type I, Osteoblast

MICROSCOPIC STRUCTURE OF DECIDUOUS TEETH FROM PATIENTS WITH HYPOPHOSPHATEMIC X-LINKED RICKETS

Klinge RF, Risnes S, Li CF, Skogedal N and Storhaug K
Department of Oral Biology, Dental Faculty, University of Oslo and National Resource Centre for Oral Health in Rare Medical Conditions (TAKO-centre), Lovisenberg Diakonale Hospital, Oslo, Norway

Irregularities in microscopic structure, particularly in the dentin, have been demonstrated in teeth from patients with hypophosphatemic rickets (Chaussain-Miller et al., 2003). The aim of this study was to further investigate the structure of teeth from patients with this disease.

The material consisted of 10 deciduous teeth from 4 patients. In 3 of the patients the diagnosis was made and treatment started around 2 years of age and in 1 around 6 months of age. Two teeth were extracted for orthodontic reasons, while 8 teeth were extracted due to pulp necrosis and abscess formation. A central longitudinal ground section was cut for light microscopy and microradiography. Of the remaining mesial and distal parts, one was prepared for scanning electron microscopy while the other was decalcified and processed for light microscopy.

The morphology of the teeth appeared normal. Hypomineralized patches in the enamel were quite frequent. The structure and degree of mineralization of the enamel were generally normal, but hypomineralized areas with large, coated crystals could be seen. The mantle dentin had a normal degree of mineralization, but the width varied. The circumpulpal dentin had a very irregular mineralization pattern in most of the teeth. Numerous calcospherites alternated with star-shaped, unmineralized areas. Closer to the pulp, the size of the calcospherites decreased. The root dentin had numerous interglobular areas, but the distribution varied and was different from that observed in the crown. The predentin layer was wider than normal and irregularities in the course of the dentinal tubules could be noted in some teeth. The cementum layer seemed to be thinner than normal, and was mostly unaffected.

The present observations indicate that the microscopic structure of all hard dental tissues is affected in hypophosphatemic rickets. The largest effect is seen in the mineralized phase of the dentin, but great variations are observed in different areas of the dentin.

Chaussain-Miller C, Sinding C, Wolikow M, Lasfargues J J and Garabédian M. (2003). J.Pediatrics. 142: 324-331.

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Keywords: tooth structure, hypophosphosphatemic rickets

IN VITRO DIFFERENTIATION OF OSTEOGENIC LINEAGE CELLS FROM HUMAN EMBRYONIC STEM CELLS

Kärner E[1], Sugars R[1], Gertow K[2], Wendel M[1]
[1]Center for Oral Biology, Karolinska Institute, Sweden;
[2]Department of Laboratory Medicine, Karolinska Institute, Sweden.

The establishment of human embryonic stem cell lines and recent progress in the area of medical research offer the possibility to understand early developmental processes, thus contributing to the improvement of strategies for regenerative medicine. Our work aims to grow human embryonic stem (hES) cells in laboratory conditions and to investigate various mechanisms by which stem cells differentiate into bone producing cells, osteoblasts.

The hES cell lines H9 (from WiCell Research Institute, USA) and HS181 (from Karolinska Institutet, Stem Cell Network, Sweden) were maintained in a medium containing KO-DMEM, KO-serum replacement, L-glutamine, amino acids, beta-mercaptoethanol and bFGF. Osteogenic cultures were maintained in KO-DMEM, 10% FBS, L-glutamine, amino acids and ,-mercaptoethanol, ascorbic acid, beta-glycerophosphate and dexamethasone. The samples were maintained for 4, 8, 15 and 25 days, and cells were assessed for morphology and calcium deposition by Alizarin Red S staining. The presence of osterix, runx2, osteocalcin, bone sialoprotein, osteopontin and collagen I were analyzed by RT-PCR. Markers for early mesenchyme, such as BMP4, and bone extracellular matrix proteins, such as osteocalcin and bone sialoprotein, were assessed by immunohistochemistry. In both the hES cell lines H9 and HS181, our results demonstrate the presence of BMP4 in the forming bone-like nodules by immunostaining. In addition we also show the in vitro mineralization of such nodules by Alizarin Red S staining and the expression of runx2, osterix and collagen I. Immunohistochemical staining reveals the presence of bone sialoprotein, known to be an early marker for osteoblast differentiation, and osteocalcin, a late marker of osteoblast differentiation. Taken together our study demonstrates the successful differentiation of two human embryonic stem cell lines into osteoblastic lineage.

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Keywords: embryonic stem cells, osteogenesis, mineralization.

INTEGRIN α11β1 PLAYS AN IMPORTANT ROLE IN MOUSE INCISOR DEVELOPMENT

Popova SN[1], Barczyk M[1], van der Bos T[2], Beertsen W[2], Gullberg D[1].
[1]Department of Biomedicine, Division of Physiology, The University of Bergen, Bergen, Norway
2Department of Periodontology, Academic Center for Dentistry, Amsterdam, The Netherlands

α11β1 integrin is a collagen receptor which is expressed in the mesenchyme during embryogenesis. We have previously shown that α11 integrin RNA and protein are expressed in perichondrium, periosteum and in ectomesenchymally derived cells in the craniofacial region. We have also demonstrated that α11 is the only collagen-binding integrin expressed in the mouse incisor periodontal ligament (PDL)(1).

α11 integrin-deficient mice are dwarfed which can partially be explained by defects in incisor structure. Upper and lower incisors of α11 ko mice are misshapen and shortened in length when compared to control mice. Other features of the tooth phenotype include reduced eruption rate of the incisors, widening of the PDL and buckling at the apical incisor end. In order to clarify possible cellular mechanisms of the phenotype we compared the ability of control and mutant fibroblasts in assays for cell attachment, cell spreading, cell proliferation and collagen lattice reorganization. Our results support a model where the periodontal ligament fibroblasts, through their collagen remodelling capacity, play a key role in incisor tooth eruption.

1. Popova SN, Rodriguez-Sanchez B, Liden Å, Betsholtz C, van den Bos T, Gullberg D. (2004) Dev Biology 270: 427

Keywords: α11β integrin, periodontal ligament, incisor

GENE EXPRESSION DIFFERENCES BETWEEN NATIVE HUMAN PULP AND ODONTOBLASTS

Pääkkönen V[1], Vuoristo J[2], Salo T[1]1 and Tjäderhane L[3]
[1]Institute of Dentistry, University of Oulu, Finland; [2]Microarray Core Facility, Biocenter Oulu, Finland; [3]Insitute of Dentistry, University of Helsinki, Finland

Background: Microarray is a method that enables comparing expression of thousands of genes between samples at one experiment. Knowledge of gene expression differences between pulp and odontoblast might give insight to the regulatory mechanisms of these spatially related but functionally diverse cells. Objective: To study gene expression differences between native human pulp tissue and odontoblasts using microarray.

Methods: Total RNA was isolated from native pulp and odontoblast samples using Trizol reagent, and Affymetrix microarray analysis was performed to both samples. The microarray used to study pulp tissue included 22 000 probe sets representing 18 400 different transcripts and variants, and microarray used for odontoblasts consisted of over 54 000 probe sets, representing 47 000 transcripts and variants. The genes expressed only in pulp tissue or in odontoblasts were divided into Gene Ontology (GO) categories using Onto-Express software.

Results: 1595 transcripts were detected only in pulp tissue and 904 were only found in odontoblasts. The transcripts could be divided into several (GO) categories according to their cellular function. One interesting category was the genes related to extracellular matrix organization and biogenesis, which included two genes found only in odontoblasts: collagen XI a1 and matrilin 4.

Conclusions: The microarray technique provides powerful tool for detection of cell-specific gene expression in dentin-pulp complex cells. For example, collagen XI a1 and matrilin 4 are found in odontoblasts but not in pulp tissue and thus could be used as marker genes of odontoblast differentiation e.g. in stem cell research.

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Key words: gene expression, pulp tissue, odontoblasts

REGULATION OF SMALL LEUCINE-RICH PROTEOGLYCAN OSTEOADHERIN BY TRANSFORMING GROWTH FACTOR (BETA)-FAMILY CYTOKINES

Rehn A, Chalk A, Wendel M
Center for Oral Biology, Karolinska Institutet, Sweden.

Osteoadherin (OSAD) is a small leucine-rich proteoglycan (SLRP) expressed exclusively in mineralized tissues. It has ultrastructurally been located to the border between bone and cartilage in metaphyseal trabeculi, calvariae, and mature odontoblasts.
OSAD has affinity for hydroxyapatite and has also been shown to bind the integrin alpha(v)beta(3). Thus, it has a role as a structural component for crystal formation and might also have signalling properties.
Although the peak expression of OSAD is found at the time of the osteoid formation, it is expressed as early as pre-osteoblasts dedicate to the mature osteoblast. As pre-osteoblasts become committed, several factors influence the process. Among others, the members of the Transforming Growth factor (TGF)beta family: BMP-2, TGFbeta1, and TGFbeta3. BMP-2 is a potent inducer of osteoblast differentiation and upregulate the key transcription factor Runx2, while TGFbeta1 and -3 inhibit the final steps during osteoblast differentiation.
Our aim was to examine how TGFbeta1, TGFbeta3, and BMP-2 regulate the expression of OSAD as cells commit to mature osteoblast. The effects of TGFbeta family cytokines on OSAD expression was examined by a luciferase assay and RT-qPCR analysis of mouse osteoblasts in culture.
We noticed a virtually complete repression of OSAD promoter by TGFbeta1 and -3, and a significant increase by BMP-2. Similar results were seen with the RT-qPCR analysis on primary cultures of osteoblasts, where OSAD transcripts were reduced by both TGFbeta1 and -3. We could also see a similar effect by BMP-2 as seen with the luciferase experiments.
In silico analysis of the OSAD promoter revealed two putative binding sites responsive to TGFbeta signalling; a BMP-2 responsive element located 200 bp upstream of the transcription start site, and a TGFbeta responsive element 60 bp upstream of the transcription start site.

Acknowledgement: the support of COSTB23 is gratefully acknowledged.

Keywords: Small Leucine-Rich Proteoglycans, Osteoadherin, Transforming Growth factor

INTERACTIONS OF DPSC AND HSG CELLS: A MODEL FOR EPITHELIAL-MESENCHYMAL COMMUNICATION

Vág J, Szlávik V, Kádár K, Varga G
Molecular Oral Biology Research Group, Department of Oral Biology, Semmelweis University and Hungarian Academy of Sciences, Budapest, Hungary

Differentiation of tooth germ involves epithelial-mesenchymal interactions. We aimed to model these interactions using cultures of mesenchymal cells isolated from adult human dental pulp and undifferentiated epithelial cells from salivary origin. Primary dental pulp stem cell culture (DPSC) was prepared as previously described from human impacted third molars (Gronthos et al. PNAS 97:13625). HSG, an undifferentiated human salivary gland ductal cell line, capable of transdifferentiating into salivary acini, was used as an epithelial source for culture. DPSC and HSG cells were cocultured on glass slides. In separate experiments DPSC and HSG cells were cultured on the extracellular matrix derivative Matrigel. DPSC grew as elongated fibroblast-like cells on glass slides. HSG cells on glass slide grew as a monolayer with cobblestone appearance. When HSG and DPSC cells were cocultured (40-40 K cells plated onto the two sides of a coverslip) the normal growth and shape of HSG cells remained undisturbed. However, DPSCs migrated toward the HSG cells, some of them reaching to the top of HSG clusters, and changed in morphology characterized by long extensions of cytoplasm and smaller, rounded soma. Matrigel induced HSG cell differentiation into acinar-like structures in the first two days, and resulted in apoptosis of the epithelial cells in 5 days. When DPSCs were cultured on Matrigel the cells rapidly underwent growth arrest and eventually died. In conclusion, coculture of DPSC and HSG cells results in dramatic changes of cell shape and migrating activity of DPSCs, while no obvious morphological change can be observed in HSG cells. The extracellular matrix derivative Matrigel induces rather different effects, selectively promoting the differentiation of the epithelial HSG cells, but not the mesenchymal DPSCs. Further studies are needed to identify the factors transmitting the above described differentiation signals for DPSC and HSG cells.

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Keywords: dental pulp stem cell, epithelium, Matrigel