features of local strain history. In contrast to adaptations that affect global (i.e., whole bone) stiffness/strength requirements, these adaptations may enhance fatigue resistance and fracture toughness for local loading conditions.

P46 W

ZINC STAINING OF MATRIX METALLOPROTEINASES AND ENDONUCLEASES IN GROWTH CARTILAGE

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A new method for Zinc histochemistry was applied to stain zinc atoms from matrix metalloproteinases and endonucleases to localize their distribution in epiphyseal plate rat cartilage. Though these zinc ions are firmly bound and essentially they are not available, drastic ammonium sulfide exposure rendered them reactive for staining. Matrix metalloproteinases were detected in chondrocytes and in extracellular matrix along the longitudinal septa before matrix calcification. A second localization was found at the resorptive limit of calcified matrix adjacent to the zone of vascular invasion. Zinc of endonucleases involved in apoptosis was stained within the nuclei in the last rows of hypertrophic chondrocytes precisely where chromatin was condensed.

P47 T

BONE MORPHOGENETIC PROTEIN-2 STIMULATES INORGANIC PHOSPHATE TRANSPORT AND MINERALIZATION IN OSTEOBLAST-LIKE CELLS

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Bone morphogenetic proteins (BMPs) play an important role in the development of bone and cartilage. BMP-2 is produced by osteogenic cells including osteoblasts and stimulates the differentiation of preosteoblasts and the activity of osteogenic cells. Inorganic phosphate (Pi) is an important element for the calcification of the bone matrix. Recent studies in cultured MC3T3-E1 cells suggest a specific role of the Pi transport system Pit-1 in initial events of matrix mineralization. The aim of the present study was to analyse whether BMP-2 regulates the expression and activity of PiT-1 and investigate the possible role of this transporter in the BMP-2-induced matrix mineralization. BMP-2 time- and dose-dependently stimulated Na-dependent Pi transport at day=6 in MC3T3-E1 cells. An effect of BMP-2 on Pi transport was detected after 3 hours. It was maximal after 6 hours and remained expressed at least 24 hours. A maximal response was obtained with 30 ng/ml of BMP-2 (2.2 fold). Kinetic analysis indicated that BMP-2 increased the maximal rate (Vmax) of the transport system but did not affect the apparent affinity for Pi. Pretreatment of the cells with either actinomycin D (2.5 microg/ml) or cycloheximide (5 microM) completely abolished the stimulation of Pi transport induced by BMP-2. Northern blotting analysis showed an increased expression of mRNA encoding Pit-1 after 2 hours BMP-2 exposure. In parallel with the stimulation of Pi transport, BMP-2 enhanced both ALP activity and the formation of mineralized bone nodules in differentiating cells.

In conclusion, the results of this study indicate that BMP-2 stimulates the expression and activity of the Pi transporter Pit-1 in osteoblast-like cells via a RNA and protein synthesis dependent process. This effect is associated with enhanced expression of bone matrix mineralization suggesting a possible role of this Pi transport system in bone matrix calcification.

P48 S

BMP RESPONSIVENESS IN HUMAN MARROW STROMAL CELLS

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INTRODUCTION: Cultured bone marrow stromal cells from various species have been shown to possess an inducible osteogenic phenotype. Interestingly, the potency of individual inducers is species-dependent. BMP has a relative potent osteogenic effect on rat and mouse stromal cells yet is usually a poor inducer of osteogenesis in cultured human stromal cells. We have been examining why the BMP effect is poor and variable in human cells.

METHODS: Human stromal cells (HMC) were isolated from marrow aspirated from femora during total hip arthroplasty. The marrow was washed to remove fat and the mononuclear cells concentrated on Ficoll-Paque (Amersham-Pharmacia Biotech). Primary cultures were established at 5×10^5 cells/cm^2. Media in primary cultures were initially changed on day 3 and, generally, every second day thereafter. Half of the primary cultures from individual samples were treated with dexamethasone (dex; 10^{-7}M), a potent inducer of osteogenesis in HMC. Just prior to confluence, first passage cultures were established at 10^4 cells/cm^2. All cultures were treated with ascorbate phosphate at 100 microg/ ml. Selected cultures were treated with BMP-2 at 100 ng/ ml. Cultures were harvested at day 6 for alkaline phosphatase (AP) assay and total RNA isolation.

RESULTS: Baseline AP activity in first passage cultures derived from dex-treated primaries was higher than activity in cultures derived from non-dex primaries. In most first passage cultures derived from non-dex primaries, BMP-2 had no significant effect on AP activity; some isolates showed modest inhibition by BMP. In first passage cultures derived from dex-treated primaries, BMP-2 significantly increased AP activity ($p=0.01$). However, the magnitude of this effect varied widely. Interestingly, BMP-2 induced noggin mRNA in first passage cultures irrespective of primary culture condition and AP level in first passage. We conclude that human marrow stromal cells possess a latent osteogenic response to BMP which can be induced by dex treatment.

P49 W

DISTRIBUTIONS OF MRNAS FOR BMP-2 AND BMP RECEPTOR IN OSTEOARTHRITIC CARTILAGE

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[Purpose] Osteophytes are neoplastic tissues made up of osseous and cartilaginous components with fibrous mesenchymal layers. The tissues often originate from the margin of osteoarthritic joints, especially at the synovio-chondral junctions. The aim of this study is to examine the involvement of BMP-2 signaling in the process of cellular differentiation during osteophyte formation in the human.

[Materials and Methods] In situ hybridization (ISH) utilizing digogigenin-labeled cRNA probes for human bone morphogenetic protein (BMP)-2 and BMP receptor IB, and immunohistochemistry (IH) with a monoclonal antibody against human BMP-2/4. To determine the phenotypes of cells, ISH using cRNA probes for human collagens types I, II and III (Col I, II, III) were also performed. Osteophytes obtained at the surgery, with consent, from 8 specimens from 6 individuals were used in this study.

[Results] BMP-2 mRNA and protein were distributed in mesenchymal cells (positive for Col I and III, negative for Col II) overlying or adjacent to apparent osteophytes, and in chondrocytes (positive for Col II) located in neo-plastic hyaline cartilage. BMP-2 was also localized in chondrocytes in fibrocartilaginous (positive for Col II and Col III) and in mesenchymal cells undergoing intramembranous ossification forming osteophyte. In mesenchymal cells although BMP-2 was synthesized by in mesenchymal cells surrounding the osteophyte, mRNA for BMPRIB were predominantly localized in chondrocytes themselves. Neither BMP-2 mRNA and protein was detected in cells in non-osteophytic cartilage. These results is summarized in a Table.

[Discussion] These results suggest that mesenchaymal cells contribute to initiate and promote formation of osteophyte via synthesis of BMP-2. Co-localization of BMP-2 and BMP receptor type IB in cells in mesenchymal layers in mesenchymal cells located in the periphery of osteophyte suggest that BMP-2 may play roles in osteophyte fromation in osteoarthritic cartilage.

P50 T

ADENOVIRUS MEDIATED BMP-2 GENE THERAPY ENHANCES BONE FORMATION IN A MURINE METAPHYSEAL BONE DEFECT

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We have investigated the capacity of bone morphogenetic protein (BMP)-2 to enhance bone healing in a metaphyseal bone defect model in the mouse. For this purpose a recombinant adenovirus (RAdBMP-2) harboring the complete coding sequence of the human BMP-2 under the control of cytomegalovirus IE promoter was constructed. RAdBMP-2 viruses were injected into the defect site in the distal metaphysis of the femur immediately after surgery. Control defects were injected with recombinant adenoviruses harboring the LacZ gene. The healing process was followed at 7, 14, 21 and 42 days using histology, peripheral quantitative computed tomography (pQCT), biomechanical testing and molecular biologic analyses. Histologically, a characteristic effect of BMP-2 was enhanced osteogenesis within the medullary cavity and periosteal chondrogenesis adjacent to the defect, particularly during the first week of healing. At two weeks, pQCT analysis revealed increased bone mineral content (BMC) in the defect area injected with RAdBMP-2 when compared with the controls. Similarly, an increasing trend was seen in the bending stiffness of the healing femur at two weeks after RAdBMP-2 injection. Analysis of the chondrogenic and osteogenic activity in the defect area by Northern analyses revealed that the mRNA levels for cartilage and bone components in defects injected with RAdBMP-2 remained essentially unchanged, indicating a balanced increase in chondrogenesis and osteogenesis. The production of human BMP-2 in defect area was demonstrated by a reverse transcription-polymerase chain reaction (RT-PCR) assay. The highest levels of recombinant BMP-2 were seen already at one week of healing. In summary, the data demonstrate the capacity of transient overproduction of BMP-2 to induce both chondrogenesis and osteogenesis. As the defect model was developed in the mouse, it can now be tested for the biological activity of other bone inducing factors both in normal mice and in transgenic mice harboring various type of gene modifications using adenovirus mediated gene transfer.