Demineralised bone matrix stimulates osteogenesis in adipose tissue–derived canine mesenchymal stem cells

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Introduction Dogs, like humans, suffer from bone disease and bone injuries that may benefit from mesenchymal stem cell (MSC)-based therapies. The aim of this study was to investigate the ability of adipose tissue-derived canine MSCs (AT-MSCs) to differentiate into bone-forming osteoblasts. In addition, we examined whether supplementation with allogeneic bone graft demineralised bone matrix (DBM) also affected MSC growth and differentiation.
Materials and Methods MSCs were isolated from the inguinal fat pads of dogs and cultured with either osteogenic media or control medium for 30 days. These media were supplemented with DBM from 3 different dogs; donors A, B and C. After this treatment period, cell number and growth patterns were examined using phase contrast microscopy and image analysis, whilst osteogenic differentiation was assessed by staining for alkaline phosphatase (ALP) activity and with alizarin red, which detects mineralisation.
Results There was a greater proliferation of cells in control versus osteogenic conditions. Nodule formation was seen in the presence of osteogenic medium, but not control medium, albeit at a low frequency. However, there was a significant increase in nodule formation when DBM supplementation was added for two (donor B and donor C) out of three DBM donors. Similarly, there was a significantly higher frequency of ALP +ve stained cells and alizarin red stained nodules (confirming mineralisation) in osteogenic medium when supplemented with donor B DBM and donor C DBM compared with all other conditions.
Discussion Decreased proliferation, nodule formation, increased ALP activity and alizarin red-stained mineralisation of nodules all suggest that the AT MSCs underwent osteogenic differentiation. However, the evidence of donor variation in DBM activity requires further study, e.g. to examine differences in content or release of bone morphogenic proteins. Nonetheless, this study has provided evidence that AT MSCs coupled with DBM have application in the treatment of bone diseases or injuries in dogs.

Comparison of protein extraction methods in tendon for mass spectrometry analysis

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Introduction Tendon injuries can occur due to sports related incidents, as result of trauma to overuse or during disease or ageing. Using proteomic techniques tendon protein profiles could be defined under different conditions. However, little attempt has been made to identify the optimum protein extraction technique to increase the coverage of tendon proteome. The aim of this study was to optimise tendon sample preparations technique for mass spectrometry analysis, which will be valuable to future tendon research studies.
Materials and Methods Tendon samples were collected from equine superficial digital extensor tendons (SDFT) that were between 11–13 years old. The samples were divided in four parts and subjected to either 4 M guanidine- HCL, 0.1% Rapigest, a combination of 4M guanidine-HCL and 0.1% Rapigest or 4M guanidine-HCL followed by 0.1% Rapigest extraction of the insoluble pellet. Soluble extracts were reduced, alkylated, trypsin digested and desalted. The proteolysed products were identified using LC-MS/MS analysis. Proteins were identified using PEAK®7 (Bioinformatics Solutions) against the Unihorse database. Progenesis QI (Waters) was used for label-free quantification.
Results A total of 230 proteins were identified using guanidine extraction, 116 proteins with Rapigest extraction, 143 proteins using combination of guanidine and Rapigest extraction and 206 proteins with guanidine followed by Rapigest extraction method. The least variability in protein solubility and in number of identifications was found in the guanidine followed by Rapigest method. In addition guanidine followed by Rapigest method also had the lowest variability in miss-cleavage compared to the other extraction methods. Label-free analysis identified significantly more cellular proteins using the guanidine method, whilst the Rapigest alone resulted in significantly more extracellular matrix proteins such as collagen type I, III, IV and V.
Discussion This study identified the optimum method with least variability for protein extraction to increase the coverage of the tendon proteome. The data in this study suggests that Rapigest mainly functions to increase the extraction of insoluble collagen proteins, however it may not be the optimal method in terms of extracting the cellular proteins. Guanidine followed by Rapigest method was the most consistent protein extraction method compared to the other methods.

TSG-6 protects cartilage and bone by modulating the activities of chondrocytes and osteoclasts: a potential therapeutic for musculoskeletal disorders

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Introduction TSG-6 is expressed during inflammation and is known to protect joint tissues from damage in mouse models of

rheumatoid arthritis, primarily via its inhibition of neutrophil extravasation. We have shown recently that the anti-inflammatory activity of TSG-6 is mediated through binding to CXCL8 thereby blocking chemokine-glycosaminoglycan interactions. Here we investigated the effects of recombinant human (rh) TSG-6 and its isolated Link module (Link_TSG6) on chondrocytes and osteoclasts and evaluated the efficacy of Link_TSG6 in rodent models of osteoarthritis (OA) and osteoporosis.

Materials and Methods Chondrocytes in 3D pellets were treated with IL-1/TNF±TSG-6 and expression of ADAMTS4, ADAMTS5 and MMP13 quantified by qPCR. OA cartilage explants were cultured with IL-1/OSM±TSG-6 and proteoglycan release measured using the dimethylene blue assay. The rat ACLTpMx model of surgically-induced OA was treated with Link_TSG6; joint damage was evaluated by macroscopy/histology after 4 weeks and pain was assessed by tactile allodynia. Human or murine osteoclasts were cultured on dentine with M-CSF/RANKL±Link_TSG6 and lacunar resorption and F-actin ring formation quantified. Ovariectomised (OVX) mice were treated with Link_TSG6 for 8 weeks; effects on bone turnover were assessed by CT and histomorphometry of long bones.

Results Link_TSG6 was more potent than rhTSG-6 at suppressing IL-1- and TNF-induced expression of ADAMTS4, ADAMTS5 and MMP13 in human chondrocytes; i.e. the aggrecanase and collagenase enzymes responsible for cartilage degradation in OA. Link_TSG6 also significantly reduced proteoglycan loss from human OA cartilage explants and reduced cartilage damage and pain in the rat ACLTpMx model. *in vitro* assays showed that Link_TSG6 treatment inhibited osteoclast-mediated resorption via potent suppression of F-actin ring formation, thereby impairing attachment of active osteoclasts to the bone matrix. In Link_TSG6-treated OVX mice trabecular bone loss was significantly supressed, with the number and activity of osteoclasts reduced at the bone surface. Importantly there was no evidence of impaired bone formation.

Discussion The chondroprotective, anti-resorptive and anti-inflammatory properties of Link_TSG6 make it a unique target for development as a biological therapy for musculoskeletal disorders. We are now investigating the mechanism of Link_TSG6 action in cartilage and bone and whether its activities are mediated through modulation of cell-matrix interactions.

Responses to altered oxygen tension are distinct between human stem cell types and those of high and low chondrogenic capacity

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Introduction Lowering oxygen from atmospheric (hyperoxia) to the physiological level (physioxia) of articular cartilage promotes mesenchymal stem cell (MSC) chondrogenesis. However, the literature is equivocal regarding the benefits of physioxic culture on preventing hypertrophy of MSC-derived chondrocytes. Articular cartilage progenitors (ACPs) undergo chondrogenic differentiation with reduced hypertrophy marker expression in hyperoxia but have not been studied in physioxia. This study sought to delineate the effects of physioxic culture on both cell types undergoing chondrogenesis.

Materials and Methods MSCs were isolated from human bone marrow aspirates, and ACP clones were isolated from healthy human cartilage. Cells were differentiated in pellet culture in physioxia (2% O_2) or hyperoxia (20% O_2) over fourteen days. Chondrogenesis was characterized by biochemical assays and gene and protein expression analysis.

Results MSC preparations and ACP clones of high intrinsic chondrogenicity (termed high-GAG) produced abundant matrix in hyperoxia and physioxia. Poorly chondrogenic cells (low-GAG) demonstrated a significant fold-change matrix increase in physioxia. Both high- and low-GAG groups MSCs and ACPs significantly upregulated chondrogenic genes; however, only high-GAG groups had a concomitant decrease in hypertrophy-related genes. In physioxia, high-GAG MSCs and ACPs produced comparable type II but less type I collagen than those in hyperoxia. Type X collagen was detectable in some ACP pellets in hyperoxia but undetectable in physioxia. In contrast, type X collagen was detectable in all MSC preparations in hyperoxia and physioxia.

Discussion Physioxia significantly enhanced the chondrogenic potential of both ACPs and MSCs compared with hyperoxia, but the magnitude of response corresponded with intrinsic chondrogenicity that varied between donors and clones. Discrepancies reported in the literature regarding MSC hypertrophy in physioxia may be explained by the use of low numbers of preparations of variable chondrogenicity. Physioxic differentiation of MSCs of high chondrogenicity significantly decreased hypertrophy related genes but still produced the hypertrophic marker protein, type X collagen. Highly chondrogenic ACPs had significantly lower hypertrophic gene levels, while type X collagen protein was below detectable levels in physioxia, emphasizing the potential advantage of these cells. Our work suggests that individual stem cell populations ought to be characterized prior to further experiments because of the potential influence of chondrogenicity on results and conclusions.

LaNt α31, a LAMA3 gene derived new protein, co-distributes with laminin in the extracellular matrix and induces early hemidesmosome maturation in corneal epithelial cells

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Introduction LaNt α31 (laminin N-terminal α31), a product of alternative splicing from the laminin α3 encoding gene