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GENETIC DETERMINANTS OF MATRIX BIOLOGY

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Tissue development is dependent upon the differentiation, function and co-ordinated interaction of various cell types. The synthesis, organisation and turnover of extracellular matrix proteins is important in providing a communication network not only between cells but also during differentiation stages within cell lineages. The identification of genes responsible for connective tissue disorders has highlighted the integral role of matrix proteins, growth, differentiation and transcription factors in tissue growth. A variety of strategies including the candidate gene approach, naturally occurring animal models and transgenic mouse technology have made possible the recent explosion in information concerning gene products involved in disorders.

Defects have been identified arising from the impaired synthesis of most matrix components. Collagens, proteoglycans, hyaluronic acid and elastin have all been implicated in genetic disorders. In most cases this leads to derangement in matrix organisation and in tissue form and function. Degradation of matrix components is also a problem as exemplified by the mucopolysaccharidoses which result from an inability to degrade glycosaminoglycan chains within intracellular organelles. Once again matrix organisation is disrupted due to the accumulation of undegraded matrix components. From the correlation between matrix gene defects and genetic disease we have been able to infer much about the role of matrix components in normal tissue development and growth.

The range of available animal models of genetic disease, both natural and transgenic, will make possible further investigations of the basic mechanism of matrix formation in tissue function, the pathogenesis of dysfunction, and will also permit evaluation of therapies for genetic disorders.

QUANTITATIVE MORPHOMETRIC ANALYSIS OF TRABECULAR BONE ARCHITECTURE IN FELINE MUCOPOLYSACCHARIDOSIS VI.

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Feline mucopolysaccharidosis VI (MPS VI) is a naturally occuring animal model of human MPS VI (Maroteaux-Lamy Syndrome). It is an autosomal recessive glycosaminoglycan storage disorder resulting from a deficiency in the lysosomal enzyme, N-acetylgalactosamine 4-sulfatase. As with human MPS VI the disorder is characterised by a wide range of clinical symptoms including growth retardation and skeletal abnormalities. In this study we compare, quantitatively, measurements of trabecular bone in normal cats, untreated MPS VI cats and MPS VI cats that have undergone various regimes of enzyme replacement therapy (ERT), with an aim to analyse the efficacy of ERT on the skeletal system.

Trabecular bone samples were obtained from the fifth lumbar vertebrae of 6 month old normal (n= 5), MPS VI (n=7) and ERT MPS VI cats (n=6). ERT MPS VI cats received intravenous infusions of varying doses of human recombinant N-acetylgalactosamine 4-sulfatase begun at birth. After sacrifice, the L5 vertebrae was routinely processed, impregnated with silver (von Kossa) and analysed at a magnification of x46 using an automated image analysis system (Quantimet 520 system, version 4.0, Cambridge instruments). Each section was divided into eight equal fields and metaphyseal trabecular bone within the boundary of each field was traced using the Quantimet system, with care taken to avoid cortical bone and growth plate. Sum totals of area, perimeter and frame area values for each field were determined and subsequently summed to obtain total area, perimeter and frame area values for each section. Using these values, parameters commonly used to describe the characteristic features of trabecular bone architecture were calculated.

Data obtained demonstrates that 6 month old MPS VI cats had a decrease in trabecular number and thickness, an increase in trabecular separation and a marked osteopenia (decreased trabecular bone volume), when compared to normal cats of the same age. MPS VI cats that had undergone ERT showed a dose dependent increase of trabecular bone volume, and as such had a lower level of osteopenia than nontreated MPS VI cats. The ERT MPS VI cats also showed a dose dependent increase in trabecular number and thickness, and a dose dependent decrease in trabecular separation. Preliminary data also suggests that frequency of dose is an important factor in determining treatment protocols.

The positive effect of ERT on skeletal development in MPS VI cats demonstrated in this study indicates that ERT may be an effective method of treatment for the devastating skeletal pathology observed in MPS VI children.

IN VITRO EXPRESSION OF MUTATIONS OF TYPE X COLLAGEN

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Type X collagen is a short chain collagen expressed in hypertrophic zone of calcifying cartilage during skeletal development and bone growth. Mutations of the type X collagen NC1 domain in Schmid metaphyseal chondrodysplasia and *in vitro* expression of mutant α1(X) implicated the NC1 domain in chain assembly (1). In these studies we use site-directed mutagenesis to produce mutations to further study the role of α1(X) domains in assembly and helix formation.

Three NC1 mutations comparable to mutations defined in patients were produced by SOEing PCR (Splicing by Overlap Extension); 1952delC; 1963del10 and Y598D. An in-frame helix deletion (amino acid residues 72-354) and a deletion of the NC2 domain (157del258) (amino acid residues 21-54) were also produced. *In vitro* expression of these mutant plasmids using a coupled cell-free transcription and translation system demonstrated that mutations of the NC1 domain all prevented the *in vitro* assembly of type X collagen chains into trimers. In addition, co-translational analysis of mutant and normal type X collagen chains also demonstrated that the mutant chains do not associate with, or interfere with the efficiency of normal chain assembly.

Preliminary studies on transiently transfected cells confirm that the NC1 mutations compromise type X collagen assembly and secretion, but detailed analysis of stably transfected cells is necessary to determine if mutant collagen assembly and secretion is totally prevented *in cellulo*.

In contrast the in-frame helix and the NC2 deletions did not prevent assembly and mutant homotrimers and mutant-normal heterotrimers were formed *in vitro*, and homotrimers in transient transfections, were secreted efficiently.

1. Chan, D., Cole, W.G., Rogers, J.G. and Bateman, J.F. (1995) J. Biol. Chem. **270**, 4558-4562