

Show thumbnails in outline**Summary**

Keywords

Introduction

Materials and Methods

Induction of Tibial Dyschondroplasia

Tissue Extraction

Total RNA Extraction and cDNA

Synthesis

Real-Time Quantitative PCR

Table 1.

Extraction of Proteins

Gelatin Zymography

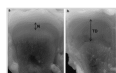
SDS-PAGE and Immunoblotting

Statistical Analysis

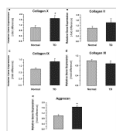
Results

Morphological Analysis of Growth

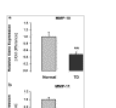
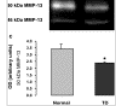
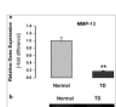
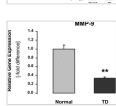
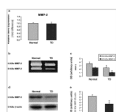
Plates from Thiram-Fed Chickens



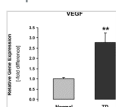
Expression of Genes Encoding Matrix Macromolecules



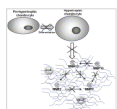
Gene Expression and Protein Levels of Matrix Metalloproteinases



Expression of the VEGF Gene



Discussion



Acknowledgments

References



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Spontaneously-Arising Disease

Expression of Genes Encoding Extracellular Matrix Macromolecules and Metalloproteinases in Avian Tibial DyschondroplasiaI. Velada^{a,†}, F. Capela-Silva[‡], F. Reis[§], E. Pires[¶], C. Egas^{||}, P. Rodrigues-Santos[†], M.T. Barros[#]^a Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal[†] Laboratory of Functional Genomics, Center for Histocompatibility, 3001-301 Coimbra, Portugal[‡] Institute of Mediterranean Agricultural Sciences (ICAM), University of Évora, 7002-554 Évora, Portugal[§] Institute of Pharmacology and Experimental Therapeutics, IBILI, Faculty of Medicine, University of Coimbra, 3000-354 Coimbra, Portugal[¶] Centre for Neuroscience and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal^{||} BIOCAN Park, 3060-197 Cantanhede, Portugal[#] Department of Health Sciences, Portuguese Catholic University, 3504-505 Viseu, Portugal

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Summary

Avian tibial dyschondroplasia (TD) is a skeletal disease characterized by disruption of endochondral bone formation. The aim of this study was to determine the expression of extracellular matrix (ECM) macromolecules and ECM-degrading enzymes [matrix metalloproteinases (MMPs)] in the growth plates of normal and TD-affected 3-week-old broiler chicks (Cobb strain). Protein levels were analyzed by immunoblotting and gelatin zymography and gene expression by polymerase chain reaction. Expression of genes encoding the ECM macromolecules (collagen types II, IX, X and XI; and aggrecan) was not altered in dyschondroplasia; however, there was down-regulation of genes encoding MMP-9, MMP-13, MMP-10 and MMP-11 in addition to reduced amounts of MMP-2 and MMP-13 proteins. In contrast, there was up-regulation of genes encoding MMP-7 and the vascular endothelial growth factor. These findings suggest that the accumulation of cartilage associated with the disease may be the result of decreased proteolysis due to the down-regulation of MMPs and not to an increased production of ECM macromolecules.

Keywords

extracellular matrix; gene expression; growth plate; matrix metalloproteinases; tibial dyschondroplasia

Introduction

Long bones develop by the process of endochondral ossification. Further elongation of long bones occurs at the level of the growth plate cartilage until the time that they can no longer grow in length. The growth plate shows a highly complex and synchronous mechanism of continuous cell proliferation, cell enlargement or hypertrophy and cell removal. Thus, the growth plate can be divided into different regions, including the reserve, the proliferative, the hypertrophic and the vascularization zones (Howell and Dean, 1992). The avian growth plate contains longer columns of cells than that of mammals and these become randomly oriented or absent from the hypertrophic and calcifying zones. More cells are found in each region of the avian growth plate, where metaphyseal blood vessels penetrate more deeply (Leach and Gay, 1987). Nevertheless, despite some structural differences between the avian and the mammalian growth plate, electron microscopical similarities suggest identical physiological control mechanisms. In fact, the process of endochondral bone growth has been reported to be functionally similar in birds and mammals (Leach and Lilburn, 1992).

The chondrocytes of the growth plate are embedded in an extracellular matrix (ECM), which supports them. The matrix is composed of ECM macromolecules, ECM-remodelling enzymes and various growth factors (Horton, 1993). Type II collagen (collagen II) is the primary collagen species in the growth plate, which also expresses collagen types IX, X and XI (Ballock and O'Keefe, 2003). Growth plate cartilage must maintain a tightly controlled balance between cartilage synthesis and degradation (Orth, 1999). Matrix metalloproteinases (MMPs), a group of ECM-remodelling enzymes, play a crucial role in ECM remodelling and degradation and are involved in the preservation of its integrity (Ortega *et al.*, 2004). The ECM also functions as a reservoir of various growth factors, which are released to adjust chondrocyte function when the ECM is degraded (van der Eerden *et al.*, 2003).

Tibial dyschondroplasia (TD) is a skeletal disease of birds in which there is impairment of normal endochondral bone formation. TD is characterized by the formation of a lesion composed of non-vascularized and non-mineralized cartilage that can extend from the epiphyseal growth plate into the metaphysis (Leach and Nesheim, 1965). The mechanisms responsible for the development of TD are still unclear. Nevertheless, several molecules have been studied in TD, including the macromolecules of the ECM, the MMPs and several growth factors. Several MMPs are expressed during endochondral ossification, including MMP-2, -3, -9, -10, -13 and -14 (Ortega *et al.*, 2004). Among these, only MMP-2, -3, -

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