Investigating the role of the RNA binding protein HuR in skeletal development

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Introduction

Osteoarthritis (OA) is characterised by cartilage degeneration and an inflammatory syndrome of the synovium, a process associated with increased levels of secreted proteases such as the matrix metalloproteinases (MMPs). MMP13, is implicated in the irreversible breakdown of the collagen type II network in articular cartilage during OA development.

HuR is an RNA-binding protein implicated in a diverse array of pathophysiological processes by modulating the stability and translational efficiency of AU-rich mRNAs. Recent data from our laboratory suggest a role for HuR in the regulation of skeletal cell differentiation and in chondrocyte MMP13 expression.

Materials & Methods

Pregnant $HuR^{+/+}$ $Cre^{+/-}$ mice were dosed with 3mg tamoxifen and 1.5mg progesterone via intraperitoneal (IP) injection at E11.5 and embryos harvested at E16.5. Embyros were fixed in 95% ETOH for 7 days, then stained for 3 days in alcian blue/alizarin red staining solution. Embryos were cleared in 1% KOH for 12-48 hours followed by a series of glycerine/KOH clearing solution. Bone mineralisation and bone density was visualised and measured using a SkyScan Micro CT scanner.

ATCD5 cells were cultured in chondrogenic differentiation medium containing human insulin for 14 days. HuR, MMP13 and other chondrogenic markers were quantified using qRT-PCR.

Results

After tamoxifen administration, one $HuR^{+/+}$ $Cre^{+/-}$ embryo displayed severe skeletal malformation when compared to WT. This included differences in the ossification of the costal ribs and spinal column, as well as differences in craniofacial structure. The chondrogenic ATDC5 cell line exhibited reduced HuR expression and increased MMP13 expression as chondrogenic differentiation progressed.

Discussion

This preliminary data suggests cartilage-specific HuR KO leads to severe skeletal phenotype during embryonic development. Creating HuR knockdown between E11.5 and E16.5 using an aggrecan promoter driven Cre Recombinase gives rise to a severe defect in spinal costal and craniofacial development. Because our HuR KO is inducible we are able to analyse the effects of HuR loss in adult mice and initial qRT-PCR data shows a reduction in levels of HuR expression in cartilage tissues in KO mice compared to their WT counterparts. The pattern of HuR expression during in vitro chondrogenesis of the ATDC5 cell line mirrors that previously observed in vivo and provides a convenient model system for investigating HuR-driven mechanisms during this process.

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