

FUNCTION OF THE RETINOID SIGNALING PATHWAY IN SKELETAL DEVELOPMENT

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INTRODUCTION: Development of the appendicular skeleton relies on a complex interplay between multiple signalling pathways to coordinate condensation and differentiation of chondroprogenitors. Although the phenotypic changes associated with chondroblast differentiation have been well characterised, much less is known about the mechanisms underlying these changes.

Vitamin-A and its metabolites, including retinoic acid (RA), are potent teratogens, which, in excess, adversely affect formation of the limb cartilages. Retinoic acid is the natural ligand for two classes of nuclear hormone receptors, the RA receptors (RARs) and the retinoid-X-receptors (RXRs). In the presence of ligand, these receptors recruit a co-activator complex, which augments gene transcription, while in the absence of ligand the receptors form a complex with nuclear co-repressors and histone deacetylase(s) to repress gene transcription. Thus, retinoid receptors have an active role in regulating gene transcription both in the presence and absence of ligand.

RESULTS and DISCUSSION: Previously, we demonstrated that mice ectopically expressing a weak constitutively active form of RAR α in the developing limb bud present with severe skeletal malformations. These defects were subsequently shown to be the result of an inhibition of chondroblast differentiation^{1,2}. Consistent with this, antagonism of RAR-mediated signalling in limb mesenchymal cultures causes an early increase in collagen type II (*col2a1*) expression that is preceded by a transient early increase in *Sox9* expression. Moreover, the activity of a reporter construct containing four repeats of a *Sox9* binding sequence from the *col2a1* gene is increased several fold in mesenchymal cultures treated with an RAR-selective antagonist or co-transfected with a dominant-negative

RAR α (dnRAR). These results reveal a close association between RAR activity and the transcriptional activity of *Sox9*. Specifically, inhibition of RAR-mediated signalling in primary cultures of mouse limb mesenchyme, results in increased *Sox9* expression and activity. This induction is attenuated by the histone deacetylase inhibitor, TSA indicating a requirement for RAR-mediated repression in skeletal progenitor differentiation.

Using the *Sox9* reporter assay, we have further delineated the pathways downstream of retinoid signaling and identified additional modulators of chondrogenesis. Of particular interest, the p38 mitogen-activated protein kinase (MAPK) signaling pathway is activated in response to expression of a dnRAR, whereas inhibition of p38 MAPKs attenuates the chondrogenic stimulation by RAR-selective antagonists. These results will be presented along with additional findings that provide a framework for understanding the role of retinoid signalling in chondrogenesis.

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