IBMS BoneKEy. 2011 November;8(11):473-478
http://www.bonekey-ibms.org/cgi/content/full/ibmske;8/11/473
doi: 10.1138/20110541

## *MEETING REPORTS*

# Chondrocytes and Cartilage Biology: Meeting Report from the 33rd Annual Meeting of the American Society for Bone and Mineral Research

**September 16-20, 2011, in San Diego, California, USA**

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More than 100 papers related to chondrocytes and cartilage biology were presented at the 2011 Annual Meeting of the American Society for Bone and Mineral Research. Signaling was the most favored topic; diverse signaling systems were studied. Another popular subject was gene regulation, including transcription factors and epigenetics. Convincing data were also presented to demonstrate the role of matrix metalloproteinase 13 (MMP13) in the pathogenesis of osteoarthritis (OA).

### Signaling

The COP9 signalosome (CSN) is a highly conserved multifunctional complex that regulates diverse biological responses (1). Jab1/CSN5 can interact with various proteins either as a monomer or as part of the CSN to regulate their functions. In order to understand the function of Jab1 in skeletal development, Chen *et al*. conditionally ablated the Jab1 gene in chondrocytes. Jab1 deletion caused increased apoptosis, defects in cell cycle progression, and accelerated hypertrophic differentiation (2). This acceleration of hypertrophic differentiation could be due to the loss of the inhibitory effect of Jab1 on Runx2 activity and bone morphogenetic protein (BMP) signaling.

Several papers investigated the role of Notch signaling. The potential role of Notch signaling in skeletal development was first reported more than a decade ago (3), but its role in regulating the differentiation of osteoblast progenitor cells and chondrocytes has been better defined only recently (4;5). Hosaka *et al*. conditionally deleted Rbpj, the mediator of canonical Notch signaling, in chondrocytes (6). Rbpj-deficient chondrocytes showed decreases in MMP13 and vascular endothelial growth factor (VEGF) expression and impaired matrix degradation. When OA was induced, Rbpj deficiency suppressed disease progression presumably by inhibiting this matrix degradation program. Zanotti *et al*. analyzed mice overexpressing the intracellular domain of Notch (NICD) that causes overactivation of Notch signaling (7). As reported previously (5), mice expressing NICD displayed severe defects in endochondral bone development. The authors found that Notch signaling inhibited nuclear factor of activated T cells (NFAT) activity, which might be responsible for the chondrocyte defects caused by overactive Notch signaling. Meanwhile, Chen *et al*. analyzed both models with gain- or loss-of-function of Notch signaling (8). As reported (6), conditional Rbpj knockout mice showed relatively mild defects while NICD overexpression caused severe skeletal defects. The skeletal phenotype of NICD-overexpressing mice was mostly rescued by simultaneous ablation of the Rbpj gene, but compound mutant mice still exhibited irregular spinal curvature and shortened tails, suggesting the existence of Rbpj-independent Notch signaling pathways in skeletal tissues.

From the perspective of developmental biology, two papers investigated the role of non-canonical Wnt signaling, and in particular Wnt5a, in chondrocyte polarity. During bone development, cartilage anlages show greater growth in the longitudinal axis. Chondrocytes change their shape according to the growth axis, but it was unknown how each chondrocyte senses positional

information. Extending their previous work demonstrating the role of the planar cell polarity (PCP) genes *Vangl1* and *Vangl2* in early embryonic laterality (9), Gao *et al*. demonstrated that the Wnt5a gradient determines the phosphorylation status and intracellular localization of Vangl2 in order to regulate chondrocyte polarity (10;11). Meanwhile, Randall *et al*. showed that Wnt5a induced formation of chondrocyte columns in pellet culture chondrocytes, a result consistent with Wnt PCP signaling contributing to chondrocyte polarity (12).

Fibrodysplasia ossificans progressiva (FOP) is caused by a weak activating mutation of the ALK2 BMP type I receptor. Chakkalakal *et al*. generated a mouse FOP model by knocking in this mutation into mice (13). Chimeric mice comprised of wildtype and embryonic stem (ES) cells carrying the ALK2 mutation developed ectopic bone formation as observed in human cases; however, unlike in humans, germline heterozygous mutation caused lethality in these mice and the animals exhibited severe growth plate abnormalities, including an increased proliferating zone and a decreased hypertrophic zone. This study provides evidence that this ALK2 mutation is causal for FOP, yet, on the other hand, illustrates the difficulty of generating animal models that truthfully reflect human genetic diseases.

Itoh *et al*. reported that glycogen synthase kinase-3 (GSK-3), a well-known regulator of Wnt signaling, targets a different signaling pathway in chondrocytes (14). Manipulation of GSK-3 altered NF-κB but not Wnt signaling in chondrocytes. It appears that GSK-3 phosphorylates RelA (NF-κB p65), which then regulates endochondral development by directly controlling expression of chondrocyte genes such as *Col2a1*. This notion was further supported by the phenotypic similarity between GSK-3 mutant and RelA conditional knockout mice.

Finally, three groups investigated skeletal abnormalities caused by neurofibromin (*Nf1*) mutations. Patients with neurofibromatosis type I (NF1), caused by *Nf1* mutations, often develop various skeletal complications including scoliosis and long bone dysplasia. Mouse models for NF1 skeletal complications were established relatively recently (15;16). At this year's meeting, Ono *et al*. reported growth plate and bone abnormalities in conditional *Nf1* knockout mice (17). Loss of *Nf1* causes hyperactivation of Ras GTPase, a major mediator of fibroblast growth factor (FGF) signaling, in chondrocytes. *Nf1* mutants displayed growth plate abnormalities similar to those of mice with achondroplasia caused by activating mutations in FGF receptor 3 (FGFR3). Interestingly, unlike mice with achondroplasia, *Nf1* mutants did not exhibit proliferation defects. Together with a previous report in which overactivity of MEK1, a mediator of Ras signaling, did not impair proliferation of chondrocytes (18), these findings suggest that Ras/mitogen-activated protein kinase (MAPK) signaling regulates chondrocyte differentiation rather than proliferation.

**Transcription Factors**

Takashima *et al*. compared gene expression profiles of chondrogenic and non-chondrogenic cells, and identified Arid5b (AT-rich interactive domain 5b) as a co-factor of Sox9 (19). *Arid5b* was co-expressed with *Col2a1* and *Sox9* in the developing limb. Arid5b-null mice showed defects in normal endochondral bone development with reduced proliferating chondrocytes. Using an *in vitro* assay, the authors demonstrated that Arid5b enhanced Sox9 function through a direct physical interaction.

The role of the Ets-related transcription factor, ERG, in mice was investigated by Ohta and colleagues (20). This group previously showed that GDF5 stimulated *ERG* expression, and that ERG overexpression in chondrocytes suppressed chondrocyte differentiation and enhanced the characteristics of joint cartilage (21). In this study, the consequences of the loss of ERG in joint cartilage were examined using joint-specific ERG knockout mice. ERG mutant mice showed normal growth but developed OA-like lesions. Parathyroid hormone-related protein (PTHrP) heterozygosity further promoted OA development in ERG mutant mice,

suggesting a functional connection between PTHrP signaling and ERG. Based on the finding that PTHrP expression was positively regulated by ERG *in vitro*, the investigators propose that ERG regulates joint chondrocyte function partly by modulating PTHrP expression. It is yet to be determined where precisely in the Indian hedgehog (Ihh)-PTHrP regulatory loop ERG is located.

**Epigenetics**

Histone modification is one of the major epigenetic gene regulatory mechanisms. There have been 130 different histone modifications identified to date (22). Among them, histone acetylation has been a major research subject. At the 2011 ASBMR Annual Meeting and in a previous paper, Bradley *et al*. demonstrated that conditional deletion of histone deacetylase 3 (HDAC3) in proliferating chondrocytes and osteoblasts using *Osterix*-Cre mice caused dwarfism and accelerated hypertrophic differentiation (23;24). In addition, mutant mice showed a decrease in extracellular matrix production and a reduction in the size of hypertrophic chondrocytes, as well as a decrease in Akt/mTOR signaling, a major regulator of protein synthesis and cell size. Based on these findings, the authors propose that the impaired mTOR signaling in HDAC3 mutants was responsible for the reduced cell size and production of extracellular matrix. These are interesting observations, but certainly require follow-up regarding the mechanism by which HDAC3 deficiency impairs mTOR signaling. In addition, the notion that mTOR signaling regulates cell size of hypertrophic chondrocytes needs further verification.

HDAC4 physically binds to MEF2 transcription factors to prevent premature hypertrophic chondrocyte differentiation (25). Previously, using an *in vitro* system, a model was proposed in which PTHrP signaling indirectly regulates HDAC4 activity to negatively control hypertrophic differentiation (26). At this year's meeting, Nishimori *et al*. provided *in vivo* evidence supporting this model (27). While the bones of heterozygous PTHrP- or HDAC4-null mice were normal, compound heterozygous mice showed significantly shorter growth plates, suggesting that PTHrP and HDAC4 regulate chondrocyte differentiation in a common pathway. In addition, the delayed chondrocyte differentiation in PTHrP-transgenic bones was normalized by simultaneous ablation of HDAC4.

Wang *et al*. investigated the role of PTHrP in thyroid hormone-induced hypertrophic differentiation (28). Using an *in vitro* pellet culture system, they demonstrated that triiodothyronine (T3) increased both the expression and activity of Mef2c, and stimulated hypertrophic differentiation. T3 treatment increased HDAC4 phosphorylation and facilitated its nuclear export. PTHrP treatment antagonized these T3-induced effects.

Nakamura *et al*. investigated the potential role of HDAC4 in neural crest cell migration using zebrafish models (29). HDAC4 knockdown resulted in craniofacial defects. These defects appeared to be caused at least in part by impaired neural crest cell migration demonstrated using *Sox10-gfp* and *foxp2-gfp* transgenic fish. Further investigation is required to determine whether this finding applies to mammals.

Histone H3 methylation regulates gene expression. The tri-methylation of lysine 27 of H3 (H3K27me3), catalyzed by the polycomb repressor complex 2 (PRC2), mediates chromatin silencing. Rankin *et al*. deleted the EED gene, an essential component of PRC2 in chondrocytes (30). Loss of H3K27me3 modification caused a proliferation defect as well as hypoxia-dependent cell death. The hypoxia-inducible factor (HIF) function in *EED*-deficient chondrocytes was reduced. This reduction in HIF function might be due to the upregulated aryl hydrocarbon receptor (AHR) that competes with HIFs for the common binding partner, aryl hydrocarbon receptor nuclear translocator (ARNT).

**Matrix**

Understanding the mechanisms of cartilage degradation is important for developing medical interventions for OA. Shen *et al*. reported that the matrix metalloproteinase MMP13 promoted OA development in OA

mouse models in which either TGFβR2 was conditionally deleted or β-catenin was conditionally activated (31). Simultaneous ablation of the *MMP13* gene prevented OA development in these models. In addition, mice lacking either MMP13 in cartilage or treated with an MMP13 inhibitor exhibited decelerated meniscal-ligamentous injury-induced OA.

Finally, Thuillier *et al*. reported an upregulation of MMP13 in chondrocytes with conditional deletion of Smad3, a major mediator of TGFβ signaling (32). Using an *in vitro* system, the authors demonstrated that Smad3 directly regulated *MMP13* promoter activity.

## Conclusion

As in other basic biomedical research fields, mouse genetics has become the dominant research method in bone and cartilage biology. In addition, thanks to the development of a plethora of specific chemical inhibitors and agonists, mouse models are becoming even more amenable to manipulation. As seen in presentations at the 2011 ASBMR Annual Meeting, sharing a common experimental system facilitated the removal of boundaries between developmental biologists, mouse geneticists and orthopedic researchers who have been studying cartilage and chondrocytes from different viewpoints. This trend will likely continue in the coming years.

**Conflict of Interest:** None reported.

**Peer Review:** This article has been peer-reviewed.

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