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MEETING REPORT

Developmental effects on mechanical signaling in the musculoskeletal system (Sun Valley 2012)

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Meeting Report from the 42nd International Sun Valley Workshop: Musculoskeletal Biology, Sun Valley, ID, USA, 5–8 August 2012

Mechanotransduction in bone and cartilage has been a common topic of presentation and discussion throughout the 40 + year history of the Sun Valley meeting. However, rarely has the meeting provided a specific session on the role of mechanical loading in the developmental phase of bone and cartilage. Appropriate mechanical stimulation during development is crucial for attaining the proper size, shape, and function of bone and joints in the newborn and beyond.[1,2] Understanding the particular cellular events that occur during developmental mechanotransduction has very practical implications for treatment of disease and injury, as many skeletal regenerative processes (for example, fracture repair, cartilage regeneration) recapitulate developmental programming.[3] Thus, elucidation of the mechanical control of bone and cartilage development is fundamental to a molecular approach to many skeletal diseases. At this year's meeting, these topics were specifically addressed in the 'Developmental Effects on Mechanical Signaling in the Musculoskeletal System' session.

An intriguing set of data were presented by Dr Elazar Zelzer from the Weizman Institute, showing that prenatal long bone growth occurs in a very asymmetric pattern, rather than simple radial expansion. The process is characterized by transient cortical buttressing in specific regions that turn out to be highly mechanically relevant. Finite element modeling and biomechanical testing of these elements demonstrate that the bones are mechanically adapted. Those observations were supported by the growth patterns in 'muscleless' mice,[4–6] which exhibit a more rounded and less adapted structure. The underloaded bones were also mechanically inferior. Another study by Dr Zelzer's group focused on developing the zebrafish as a model for perturbed mechanical signaling during development. Zebrafish embryos were paralyzed via chemical means, and changes in pharyngeal chondrocyte intercalation were noted. Mechanical signaling in these cells was dependent on β-catenin. Another recent development from Dr Zelzer's lab relates to the mechanical influence on the formation of sesamoid bones. Using the patella as a model of sesamoid developmet, Dr Zelzer showed that the patella begins as part of the femur, and essentially 'pinches off' from the developing distal femur during limb formation. Reduced mechanical loading to the lower limb prevents the patella from separating from the distal femur, and consequently, no patella is formed. The mechanisms of this activity are unclear, and it remains unknown whether this is a general mechanism of all sesamoid bones, or if the process is particular to the patella/knee. A major discussion point following Dr Zelzer's presentation was related to how these mechanical influences might be harnessed therapeutically in stem cell reprogramming.

Dr Andrew Pitsillides from the Royal Veterinary College further covered the developmental aspects of limb and joint formation. A very provocative theme of his data presentation centered around the concept embraced by some researchers that the limbs might form as a single structure that subsequently divides into separate skeletal elements with intervening zones where joints (synovium, articular cartilage, supporting tissues) then develop. This idea is in stark contrast to the more conventional view of limb element formation, where individual skeletal elements emerge independently and separately, and subsequently undergo processes that generate joint features to promote articulation of adjacent elements. Dr Pitsillides also presented some cell-lineage tracing studies, which sought to identify whether subsets of the developing joint cell population are specifically destined to form articular chondrocytes, or whether there is a general pool of cells that is used for formation of the different joint structures. Finally, some mechanically modified cellular signaling studies were presented, implicating constitutive activation of the MEK-ERK, $p38^{MAPK}$ and inducible cyclooxygenase pathways in the local synthesis of the hyaluronan-rich extracellular matrix, a process that appears central during joint cavity formation. The discussion of Dr Pitsillides' presentation focused on insights into new ways of thinking about osteoarthritis and its treatment, particularly in light of the recently identified specific subpopulation of joint surface progenitor cells from which articular chondrocytes appear to arise.

A series of technical advances in aiding the understanding of bone and joint developmental biology were presented by Dr Paula Murphy from Trinity College Dublin. Their work has focused on an optical projection tomography technique to visualize emerging tissues in 3D in the developing embryo, analyzing for example the changing 3D morphology of the chick

knee joint in detail in the presence and absence of muscle contraction. These changes in shape were compared to changes in local mechanical strain generated from finite element models of the same knee joints, to patterns of cell proliferation and to expression patterns of genes implicated in joint development. Distinctive changes in the shape of the rudiment termini, patterning of the emerging tissues of the knee joint and gene expression were described in the absence of muscle contractions. Interestingly, the intercondylar region of the distal femur was identified as a highly mechanosensitive area and could prove to be a standard model for future investigations of developmental disuse. Dr Murphy's work has also incorporated high-throughput sequencing of transcripts extracted from developing humeri of 'muscleless' mouse strains, which have yielded a subset of genes that show significant association with disuse. These data are being analyzed on a genome-wide level to reveal the biological processes disturbed with a view to uncovering the molecular mechanisms involved in mechanoregulation of this particular *in vivo* system. Moreover, forelimb and hindlimb expression patterns of selected genes yielded interesting differences in spatial patterns. The expression studies are currently being followed up with laser capture micro-dissection techniques applied to limb bud sections in order to identify mechanically induced transcriptional changes that are associated with different cell types in the developing limb.

The role of parathyroid hormone-related peptide (PTHrP) in enthesis modeling was presented by Dr Meina Wang, one of the conference's ASBMR Harold M Frost Award winners. Dr Wang used the Scleraxis-Cre transgene to recombine floxed PTHrP alleles in mice, resulting in PTHrP deletion in ligament and tendon insertion sites (entheses). Dr Wang found that the tibial medial collateral ligament enthesis failed to migrate during linear growth in PTHrP cKO mice. Instead, there was a large traction tuberosity formation at the enthesis site, accompanied by fibrochondrocyte formation, and even mineralized medial collateral ligament in adult mice. This effect was largely due to impaired osteoclast activity resulting from local PTHrP deletion. Other fibrous entheses in the PTHrP cKO mice presented a similar phenotype due to impaired osteoclast activity. Pertinent discussion points for this presentation related to whether the mutant mice exhibited impaired mechanical properties of the ligaments, and whether the mice might exhibit evidence of joint contracture as they age.

Conflict of Interest

The author declares no conflict of interest.

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