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Summary

Hylonome

Summary – Tutorial: Genetically Modified Animal Models to Study Bone and Cartilage

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The availability of techniques to introduce genetic alterations in mice has greatly facilitated the ability of bone scientists to identify the roles of specific genes in the *in vivo* setting. Because the specific shapes and microarchitecture of bone closely determine the function of bone, the use of intact bones in these studies has been a particularly important advance. In this tutorial, three experts reviewed the current methods for creating and characterizing mice useful for studying different aspects of skeletal biology.

The objectives of the tutorial were to: (1) Understand the current methodologies for knockout and transgenic expression of genes and know which models are available. (2) Understand the limitations of the current methods for phenotyping mouse bone. (3) Have a working understanding of the kinds of studies that have been done using genetically modified mice.

In the first session, Dr. Henry Kronenberg reviewed contemporary methods for creating mice with specific genetic alterations. Standard transgenic mice are relatively easy to make, but because of random sites of gene insertion, can unintentionally disrupt an endogenous gene or give rise to variable levels of expression of the transgenes. Thus, in all transgenic settings the most important step is to confirm that the transgene's control region provides the cellular specificity desired to ensure the validity of the transgene expression on bone phenotype. Gene knockout/knockin mice take advantage of murine embryonic stem (ES) cell technology. Embryonic stem cells are useful because they can populate all the tissues of the embryo enabling the study of single gene modifications on bone. Limitations of the methodology include the possibility that multiple genes provide the function of the studied gene (redundancy). Consequently, when the gene is knocked-in or out, the effects may be undetectable or alternatively lethal. For these reasons, techniques have been developed to knock genes out only from certain cells and at certain times. The cre/lox system enables genes to be removed conditionally, i.e., from particular cells, by making transgenic mice that express nucleases only in specific cell types, such that the gene of interest will be removed only in the desired cell type. However, these approaches must be carefully validated since the unsuspected expression of cre can lead to developmental problems in bone that are not due to the adult expression (or lack thereof) of the gene of interest. Thus the mouse's phenotype could be due to indirect effects. Further refinements of this technology depend on strategies to limit the time of expression of the cre molecule. These gene switches are desirable because they enable transient gene modification.

In the second session of this tutorial, Dr. David Rowe presented methods for establishing and verifying osteoblast progenitors *in vivo* and *in vitro*. The approach relies on transgenic mice bearing osteoblast-specific promoters linked to green fluorescent protein (GFP) that activate at defined levels of development within the bone lineage. Critical to this strategy is the demonstration that the cells identified by a GFP signal represent a cell with reproducible properties that meet a defined level of development. One caveat with this approach is that an expression pattern is associated with a specific transgenic line. Therefore, extensive validation of transgene expression is essential to ensure that it replicates the endogeneous gene that is being reported. The use of different fluorescent versions of GFP, such as GFPcyan, permits multiplexing the promoter-GFP colors which increases the power of a histological image of bone. Multiplexing is particularly useful in interpreting a transplantation experiment in which the donor and recipient carry a different color

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marker driven by the same promoter. FACS analysis of the GFP positive cells, coupled with microarray analysis of gene expression, allows identification of patterns of genes associated with differentiated osteoblast populations and the potential to understand the concerted action of a cell or tissue. However the interpretation of a regulated gene list can be clouded when the genes are widely expressed and the sample is composed of many cell types or levels of differentiation. The GFP strategy has the potential to overcome this limitation of microarray by providing a well-defined and relatively homogeneous sample by FACS sorting.

The final session, led by Dr. Russell Turner, reviewed the current methods for skeletal phenotyping with special reference to applications for mouse bone. Biochemical measurements of serum and urine markers for bone turnover are of limited value because they primarily originate from cortical bone and mainly reflect changes in bone growth. The most widely used method for determining bone mass is Dual Energy X-ray Absorptiometry (DEXA) which gives a projection of the bone cross-sectional area and is used to calculate bone mineral density (BMD). BMD is usually reported (and often interpreted as) an index of bone density or bone mass, but is neither. It can provide only the adjusted bone mass following adjustment for size. Nonetheless, DEXA represents a very useful tool to perform longitudinal bone mass measurements at multiple skeletal sites. It has limited ability to resolve separate bone compartments (e.g., cancellous from cortical), and is generally not useful for cancellous architecture or bone turnover. The more recently available high resolution micro CT scanners provide bone mass, volumetric bone mineral density and the ability to distinguish between cortical and cancellous bone architecture. However, because of the small amount of cancellous bone in the mouse, even the highest resolution scanners with voxel sizes of 9 microns may be of limited value for measurement of microarchitecture. *In vivo* CT scanners are becoming popular because they enable serial measurements but have the limitation of delivering significant radiation that can alter bone cell function (DNA damage/apoptosis, cytokine production). To circumvent this problem, one can decrease resolution (radiation dose), reduce the number of scans, alternate between limbs, and include appropriate controls. Bone histomorphometry remains indispensable for phenotyping mouse bone because it provides critical information such as bone cell number, formation/mineralization rate, and osteoid volume that cannot be obtained by the less invasive measures discussed above. It also provides two-dimensional architecture and the ability to measure gene expression, cell birth and death, and finite element analysis. The main drawbacks include the time and expense associated with the performance of the histomorphometry and the variation introduced by sampling.