A Novel Murine Model Of Adynamic Bone Disease (ABD)

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Introduction: The etiology of Adynamic Bone Disease (ABD) is poorly understood but the hallmark of ABD is a lack of bone turnover (1). ABD occurs in renal osteodystrophy (RO) and is suspected to occur in elderly patients on longterm anti-resorptive therapy (i.e. bisphosphonates). A major clinical concern of ABD is diminished bone quality and an increase in fracture risk. Current animal models of RO-ABD are complicated and involve surgical intervention to produce the adynamic bone phenotype (2, 3). Secondary hyperparathyroidism is often a complication of experimental renal ablation, and interventions to circumvent this problem often become confounding issues in renal failure models of ABD. Thus, there is a need for an alternate approach to modeling ABD without the complications of renal ablation. To our knowledge, experimental animal models for ABD other than RO-ABD have not been developed or studied. There were two main objectives in this study: 1) to develop and validate a mouse model of ABD without the complications of renal ablation, and to characterize changes in bone quality in ABD; 2) to investigate how bone quality changes with age in our mouse model of ABD to gain insights into the mechanisms of disease.

Methods: Tissue-targeted transgenic expression of herpes simplex virus thymidine kinase (HSV-tk) has been used to conditionally ablate specific cell populations (4, 5). In our mouse model, expression of a truncated version of the tk gene (\(\Delta tk\)) was driven under a 2.3-kilobase (kb) fragment of the rat type 1 collagen al (Col1a1) promoter (6). To create the adynamic bone condition, female Col2.3\(\Delta tk\) (DTK) mice were treated simultaneously with ganciclovir (GCV) and pamidronate to mimic loss of bone turnover. Ganciclovir treatment ablated osteoblasts (OB) to induce progressive bone loss while pamidronate inhibited bone resorption by osteoclasts (OC). Four groups of animals (4-months of age) were used to characterize bone quality in ABD: DTK Controls, No Formation Controls, No Resorption Controls, and an A Dynamic group. After a 6-week treatment period, animals were sacrificed and bones were harvested for analyses. Bone quality assessments were conducted using established techniques including bone histomorphometry, tartrate-resistant acid phosphatase (TRAP)-staining, backscattered electron imaging, dual energy x-ray absorptiometry (DXA), microcomputed tomography (\(\mu\)CT), and biomechanical testing. In the second part of the study three age groups were studied: 4-, 8-, and 16-months of age, representing young, middle-aged, and old mice.

Results: Histomorphometry confirmed the cellular hallmarks of ABD in our mouse model. Bone formation was near complete suppression in the No Formation and A Dynamic groups. Inhibition of bone resorption in the A Dynamic group and No Resorption Controls were confirmed by TRAP-staining. Pamidronate treatment increased BMD in the No Resorption Controls and the A Dynamic animals but there was no statistical difference in BMD between the A Dynamic group and DTK Controls. In addition, animals in the A Dynamic and No Resorption groups showed a decrease in heterogeneity of mineral distribution and a corresponding increase in the maximum peak height of the density distribution, with a shift in the degree of mineralization towards a more hypermineralized profile compared to DTK Controls. This data confirmed successful replication of the adynamic bone condition in a mouse.

In the second part of the study, histomorphometry and TRAP-staining analyses confirmed a natural decline in both bone formation and resorption with age in DTK Controls, whereas bone turnover was severely blunted at all ages in the A Dynamic group. There were no statistical differences in bone mineral density (BMD) between DTK Controls and A Dynamic mice at all ages, but ageing A Dynamic mice had better preserved trabecular bone volume and microarchitecture compared to DTK Control mice. However, a normal BMD and improved microarchitecture in ageing A Dynamic mice did not result in improved vertebral mechanical properties (Figure 1). Three-point bending of the right femur showed aged ABD mice required less energy to failure than younger ABD mice and this decrease was only due to changes in post-yield strain capacity (Figure 2).

Discussion: Similarity of our mouse model to the human condition was confirmed using the same histological techniques that are used to diagnose ABD in human RO. Our mouse model also presented the same skeletal phenotype as the human condition (7). Little is known about the effects of metabolic bone disease on bone toughness. Although our ageing ABD mice showed anti-resorptive treatment had a protective effect on BMD and trabecular microarchitecture, the preservation of these properties did not translate into improved biomechanical properties in the adynamic bone condition. Despite a normal BMD, the bones of ageing ABD mice were less tough than that of Control mice, most notably in the cortical compartment, and this decrease may be due to changes beyond the mineral phase. This finding may reflect observations in the clinical setting where patients on longterm anti-resorptive therapy experience atypical fractures in the subtrochanteric region in the femur shaft. Additional studies are being conducted to further elucidate changes to both bone material and structural properties in the adynamic bone condition and to identify mechanisms that contribute to reduced bone toughness in ABD. Our mouse model may be useful in the investigation of the mechanisms involved in fractures occurring in elderly patients on anti-resorptive therapy who have very low
bone turnover.

Significance: Our approach is the first model of ABD that uses pharmacological manipulation in a transgenic mouse to mimic the bone cellular dynamics observed in the human ABD condition. Our novel mouse model will help understand changes to bone quality and fracture risk as a consequence of low bone turnover. More importantly, our data suggests that current clinical diagnostic technique (i.e. bone mineral density testing) is not a good metric for bone health in ABD.

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References:
Figure 1: Ageing ABD animals maintained trabecular volume and microarchitecture (left panel) but not bone mechanical properties (right panel).
Figure 2: Aged ABD animals (16-month) showed a loss of toughness in cortical bone despite a normal BMD.